University of Alberta

Biochemical markers of bone turnover in middle aged men

by

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ABSTRACT

Biochemical markers may capture the imbalance between bone formation and resorption influencing osteoporosis. They may allow quantitative evaluation of rates of bone loss, and thereby identify persons at risk for osteoporosis. Determinants of marker levels may also provide insights into factors influencing bone turnover.

From the anthropometric components studied in a cross-sectional study of men 40 to 70 years of age, fat-free BMI had the highest association with the markers and spine BMD ($r = -0.21$ to -0.42, $p<0.05$), while body fat did not correlate with the BMD measures, indicating that fat-free parameters reflecting bone and muscle mass play a more important role in bone metabolism than body fat in men.

A classic twin study of 147 monozygotic and 153 dizygotic male twin pairs from the same population-based cohort was conducted to examine for the first time in men, genetic and behavioral factors that could influence markers originating from type I collagen. The findings supported a dominant role for heredity, with additive genetic effects explaining two thirds of the variance in the bone resorption markers in men. The genetic variance in bone markers was largely independent from the other anthropometric and behavioural co-variates studied.

NTx was a better indicator of current BMD status than PINP marker or the ratio of PINP to NTx in men, with the highest association with BMD at the sites tested ($r = -0.20$- -0.29). A longitudinal study further assessed whether markers of bone formation (PINP and PICP), and of bone resorption (ICTP), are predictive of changes in lumbar spine and femoral neck BMD over a 5-year period, and the NTx marker ability to explain

the variance in BMD change over the past 5 years in men 35–69 years old. NTx was the only marker to correlate significantly with BMD changes at the femoral neck (r = -0.21), but not at the spine. Degenerative features at the vertebrae such as osteophytes, endplate sclerosis and fatty degeneration appeared to affect the overall assessment of bone mineral density by DXA at the spine and femoral neck, and dilute the association between markers and bone mineral density measurements (DXA).

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To my grandmother, Buna (Rosu Elisabeta)

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CHAPTER 1: INTRODUCTION, REVIEW OF THE LITERATURE AND OBJECTIVES

OSTEOPOROSIS

Osteoporosis was defined as a systematic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, leading to increased bone fragility and susceptibility to fracture.[1] Inspite of a number of problems regarding the interpretation of this conceptual definition and its adaptation for clinical use, the definition has survived the rigors of a later consensus development conference (1993) [2] and has been used internationally until the definition was significantly changed at a recent National Institutes of Health (NIH) consensus development conference (2001). [3] NIH defines osteoporosis as a "skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture". It is specified that compromised bone strength reflects both bone density (determined by peak bone mass and amount of bone loss) and bone quality (including bone architecture, bone turnover, microfractures and mineralization). It was also suggested that bone quality can be assessed by evaluating the status of bone metabolism through bone turnover markers. [4]

An estimated 1.4 million people suffer from osteoporosis in Canada alone. One in 4 women and 1 in 8 men over the age of 50 has osteoporosis, but the condition can appear at any age. [5,6] The incidence of osteoporosis may be underestimated because men are less likely than women to have a bone density scan.[7] The occurrence of fractures is the main problem secondary to osteoporosis, and often translates into irreversible damage and loss of bone function. [8] According to the World Health Organization (WHO) [9] the number of hip fractures due to osteoporosis is expected to rise over three fold from 1.7 million in 1990 to 6.3 million in 2050. Therefore, this is a major public health problem affecting patients' quality of life and causing substantial medical costs. The costs of treating osteoporosis and the consequential fractures are estimated to be $1.3 billion each year in Canada, [6] and are expected to rise up to $32.5 billion by 2018 in Canada. [6]

As stated by NIH, [3] osteoporosis is not an age or gender-dependent condition. About one third of all hip fractures occur in men, and the prevalence of spine fracture is about half that of women. [10] Moreover, the mortality associated with hip or spine fracture seems to be higher in men than in women. [10,11,12] Yet, while there has been a large amount of research focused on women, there is a paucity of information in the scientific literature concerning osteoporosis in men.

DIAGNOSIS OF OSTEOPOROSIS

Although compromised bone strength is a known predisposing factor for osteoporosis, there is no accurate measure to date to assess overall bone strength. [3] The diagnostic criterion for osteoporosis was originally based on dual-energy x-ray absorptiometry (DXA). However, it is still not clear how to apply the WHO [9] diagnostic criterion (a T score of at least 2.5 SD below the mean for young adult women) to define diagnostic thresholds using different techniques, different skeletal sites, or different populations (e.g. men, children or other ethnic groups). [3] Although a serious and frequent condition in men, the apparent lower osteoporosis prevalence in men as compared to women may be in part due to a greater bone size, greater peak bone mass due to pubertal bone accrual, the absence of an abrupt decrease in sex hormones similar to menopause and a shorter average lifespan. [13] Although some authors have studied ethnic and gender differences in bone mineral density (BMD) and bone turnover, [14,15] strict diagnostic criteria for osteoporosis in men and non-white women are still lacking. Therefore, it was suggested that the diagnosis of osteoporosis in men under age of 50, premenopausal women and children should depend on risk assessment rather than the assessment of a T-score alone. Also, it was recommended that a Z-score and specific data bases should be used for different ethnic groups or children. [16] Osteoporosis Canada has also recently recommended that fracture risk should be assessed by using the individual's 10-year absolute fracture risk based on age and BMD rather than BMD alone. [17]

RISK FACTORS

It was suggested that osteoporosis is a polygenic, multifactorial disease with genetic, hormonal, environmental and nutritional determinants. [18,19] An official report of the International Society for Clinical Densitometry, [16] classified the risk factors for osteoporotic fracture into categories of major, additional, medical conditions and medications. The "major risk factors" include: history of fracture (personal and first degree relative), low body weight, smoking and glucocorticoid therapy, while "additional risk factors" include: vision deficit, estrogen deficiency, dementia, frailty, recent falls, low physical activity, low calcium intake and high alcohol consumption. [16] As stated by NIH [3] the predictive factors for low bone mass (excluding secondary causes of osteoporosis) include "female sex, increased age, estrogen deficiency, white race, low weight and body mass index, family history, smoking and history of prior fracture". The most common secondary causes associated with osteoporosis in men are hypogonadism, alcoholism and glucocorticoids use (accounting for 30-60% of osteoporotic cases in men) and hypoestrogen, anticonvulsives, glucocorticoid and thyroid hormone therapy in women (accounting for approximately 50% of cases in perimenopausal women). [3]

Genetic and constitutional factors

Twin and family studies have indicated that genetic factors account for up to 80% of the interindividual variation in BMD in both men and women.[20, 21, 22, 23] Family history is considered a strong predictor for osteoporotic fractures for white women. [24] Family-based studies also yielded strong heritability estimates for BMD in males. [25,26] Kannus (1999) [27] suggested that an inherited susceptibility to osteoporotic fractures exists in males, with a 4-fold higher concordance for fractures in monozygotic twins as compared to dizygotic twins.

Also, many limitations of epidemiological studies can be overcome by the study of twins. Twins are uniquely matched for age, sex and multiple confounding variables that can be controlled. Therefore, twin studies enable a powerful design using a relatively small sample. Monozygotic (MZ) pairs share genetic effects fully (all genes) but also have common environment during childhood and adolescence. In contrast, dizygotic (DZ) pairs share on average half of their segregating genes, but have a common environment equal to that of MZ pairs on average. Therefore, a greater similarity of MZ pairs compared to DZ pairs provides evidence for genetic effects in a trait. The extent to which MZ pairs are more than twice similar than DZ pairs permits approximation of additive from dominance effects. The classic twin model is used to estimate the contribution of additive and dominance effects of genes, as well as the contribution of common and unique environment in a specific trait. The total phenotypic variance in a trait can be decomposed to additive effects of genes taken singly and added over multiple loci (A), dominance effects of genes interacting within loci (D), common environment shared by family members (C) and unique environment not shared by family members (E).

The results of twin and family studies prompted the search for candidate genes for different aspects of osteoporosis. The timing of puberty is an important determinant of peak bone mass for men as it is in women. A history of delayed puberty was found in 2-3% of men with idiopathic osteoporosis.[7] Although men do not undergo the same abrupt menopause as women, the bone histological findings in hypogonadal men are comparable to those found in women with estrogen deficiency. [28] After recent studies that found estrogen deficiency as the probable leading cause of osteoporosis in men and women, [29,30] genes involved in sex steroid metabolism, such as aromatase, estrogen receptor alpha (ERα), ERβ and androgen receptor (AR) were studied as possible factors associated with male osteoporosis. [31,32] However, it is unclear whether the same genes influence bone mass in males and females.

The candidate genes found to be implicated in *male* osteoporosis include: vitamin D receptor gene (VDR), collagen type I alpha 1 gene (COL1A1), insulin growth factor I gene (IGF-I), aromatase (CYP19), ERα and AR genes. [19] The genes associated with osteoporosis in *women* include: α2-HS-glycoprotein (AHSG), ER, interleukin 6(IL6), COL1A1, COL1A2, VDR, transforming growth factor β1(TGFβ1) and apoprotein E (APOE). [22] Although recent studies were conducted to identify allelic variants in several genes that may be implicated in osteoporosis, the chromosomal locations, interactions and effects are not well defined. [22]

The factors that mediate the genetic susceptibility to fracture may differ from those that influence BMD. Determinants of fracture risk that are thought to be under genetic control are: the rate of bone turnover, age-related bone loss [33] and the geometry and microstructural integrity of bone. [34] Therefore, biochemical markers of bone turnover may provide explanations for the mechanism of bone loss that cannot be captured by BMD measures. Several studies of biochemical markers showed that bone turnover is heritable in women. [33,35,36,37] Genetic factors contributed significantly to the interindividual variance of bone formation markers like osteocalcin, [33,35] serum propeptide of type I collagen (PINP/PICP), [35,36] and bone specific alkaline phosphatase (BAP), [37,38] as well as bone resorption markers like carboxyl-terminal telopeptide of type I collagen (ICTP) [36] and the amino-terminal type I collagen peptide (NTx) (Garnero et al., 1996). [35] Some candidate genes were also studied in association with the markers NTx and PINP in men, like estrogen receptor gene, ER (Xbal and Pvull polymorphisms) and AR (CAG repeat polymorphism). [39] The AR gene CAG repeat polymorphism was not found to be associated with PINP, NTx, [39] PICP and ICTP. [40] Higher levels of PICP, high PTH and low BMD found in women carrying the Px haplotype of the ERα gene and s allele of COLI A1 gene suggested possible interaction between ERα and COLI A1 Sp1 polymorphism and resulting in altered production of the α1(I) chain (the protein product) and greater risk of low BMD and high bone turnover. [41] Because of the complex polygenic etiology of osteoporosis, some of the polymorphisms studied might be only a modest component in bone turnover variation or might be masked by other factors, including environmental influences. Therefore, results of possible candidate genes for biochemical markers need to be replicated in further studies. However, through the estimation of genetic and environmental influences on specific traits, twin studies provide a starting point for the search for candidate genes and further tests to confirm whether scores on a trait are associated with a specific allele.

Other *constitutional factors* that are thought to influence bone mass are: race, gender and weight. Afro-American populations have a higher bone mass than Caucasians and Asians [42,43] and lower fracture incidence. [24] Also, a gender difference was observed, as men have denser bone mass than women. [44] Low weight and lean mass were found to be correlated with bone mass in men. [45,46] Men with lower weight and lean mass had significantly lower femoral neck BMD.[47] Lean body mass in men was also positively correlated with BMD at the spine and total body BMD, while body fat mass was not correlated with lumbar spine and total body BMD. [48] Greater lean mass, more intense physical activity and less body fat might also be reasons for the relationship between low bone mass and BMD in men.

Environmental and Behavioral factors

Immobilization and physical activity

Frequently a natural consequence of a disease, immobilization could contribute to secondary causes of osteoporosis. The bone mass loss during immobilization might be secondary to a loss of muscle mass and strength and resultant forces across bone. [49] Krolner et al. (1983) [50] studied the skeletal effects of therapeutic bed rest and re-ambulation in a consecutive series of 34 hospitalized patients (aged 18-60 years). The authors observed a mean decrease in lumbar spine bone mineral content of 1% per week during immobilization, and a bone mineral gain following re-ambulation. The restoration of lumbar spine bone mineral content was nearly complete after 4 months (Krolner et al., 1983). [50] However, another author suggested bone loss could continue for about 6 months and as much as 40% of total skeletal mass might be lost during the period. [51] In the case of elderly patients, the same amount of bone may be lost in 1 week of immobilization as in 1 year of uncomplicated osteoporosis. [51]

Scheld et al. (2001) [52] observed that the level of serum intact osteocalcin was already reduced after two weeks of bed rest and that during remobilization both bone formation (osteocalcin and BAP) and bone resorption (NTx) markers reached the baseline values. Osteocalcin presented low values for the entire immobilization period of 14 weeks, stressing the reduction of bone synthesis. In the same study, NTx level was 69% higher during week 10 and 14 compared to values recorded before the resting period, suggesting increased osteoclastic activity. The authors observed significant association between changes in osteocalcin level and calcium excretion/24h ($p<0.001$), nitrogen balance ($p<0.025$) and phosphorus excretion/24 h ($p<0.001$). Also, significant association was assessed between nitrogen balance and NTx excretion. Therefore, the noted bone mass loss following prolonged immobilization might also be due to a decrease in total muscle mass.

The effect of physical activity on bone mass has been partially explained by the fact that muscle is the primary producer of mechanical forces responsible for adequate formation of bone mass. [53] Some authors suggested that age-related bone loss that occurs in both men and women later in life is related to the decline in muscle mass with advancing age. [51]

The effect of training on BMD and bone turnover markers was noted by Vincent and Braith (2002), [54] who observed an increase in BMD of the femoral neck ($p<0.05$) as a result of high intensity exercise (2%) and an increase in osteocalcin of 25% for the low intensity exercise and 39% for the high intensity exercise group ($p<0.05$). A significant effect of exposure to physical activity on BAP level was demonstrated only for the high intensity exercise group (7.1% increase, $p<0.05$). BMD of the femoral neck also increased significantly ($p<0.05$) as a result of high intensity exercise (2%). Therefore, it was suggested that the impact of different physical exercises activities varies with respect to the intensity level of training and the nature of the sport training loads, and that the association with ground reaction forces may be more important determinants in BMD than muscle contraction. [55] Although no significant differences were found in radius BMD values among women with less than 10 postmenopausal years from different sport intensity groups (high-impact to arm, low impact to arm and swimming), the BMD values in women with more than 10 postmenopausal years were higher in high and low impact sport groups compared to those in the control group without regular exercising. [56] A correlation between NTx marker of bone resorption and low impact physical activities like leisure time and work physical activity ($r=-0.25$- -0.32) was also found by Iwamoto et al. (2002) [57] in 70 men 48-85 years old. NIH (2001)[3] concluded that exercise in childhood and adolescence showed inconsistent associations with bone mass later in life.

Nutritional factors

An NIH Consensus Development Panel (2001) [3] suggested that balanced and good nutrition are essential for bone development. Calcium intake, vitamin D, balanced nutrition, dietary protein, caffeine, sodium, phosphorus, and regular exercise have been cited as factors implicated in the development of bone tissue. [3,51] Yet, the dietary factors implicated in osteoporosis etiology are poorly validated. A balanced nutrition was found to decrease the risk of complications and mortality in elderly suffering from osteoporosis, while malnutrition was associated with a higher risk of falling.[58]

Dietary calcium is currently considered the most important nutrient for achieving peak bone mass and for osteoporosis prevention and treatment, while vitamin D is essential for calcium absorption. Other nutrients like high dietary protein, phosphorus, caffeine, and sodium could affect calcium balance.[3] A study conducted by Johnston et al. (1992)[59] showed a 3% greater increase in bone mass over 3 years of follow-up in the group of young twins assigned to calcium supplement, compared with those assigned to placebo. Although vitamin D deficiency is a well-recognized cause of fracture, due to a decline in renal function that causes secondary hyperparathyroidism that occurs with aging, there is no clear evidence that it is associated with osteoporosis.[51] Undernutrition and prolonged vitamin D deficiency during childhood delay puberty, which is a known risk for osteoporosis. Therefore, the most important effect of calcium and vitamin D nutritional intervention is thought to be obtained in children, before puberty, when sex hormone-dependent bone growth is not occurring.[3,60] However, adolescents' diet requires special attention, as frequent low intake of calcium and vitamin D has been observed in this age group.[3]

Caffeine may have a contributory role in osteoporosis, due to increased urinary excretion of calcium,[61] but data are not convincing.[3,62] The consumption of tea, which also contains caffeine, was associated with a decrease in hip fracture incidence, and daily milk consumption was found to offset for the effect of coffee.[62] Alcohol abuse has been identified as a risk factor for osteoporosis, including in men.[63] However, it cannot be excluded that alcoholism might be accompanied by a poor nutrition. A toxic effect of alcohol on bone formation and an association with osteoporosis and fractures were found in women,[64] while moderate alcohol consumption has been associated with higher bone mass[65] and reduced bone loss.[66] A recent NIH Consensus Panel (2006)[3] concluded that the use of alcohol and caffeine was inconsistently correlated with bone mass decrease and that the effect of caffeine, high protein, phosphorus or sodium intake might not be important if the calcium intake is adequate.

Although calcium may not play a critical role in bone mass for persons on a mixed diet, low dietary intake could determine an increase in bone turnover that appears in osteoporosis.[51] In a study of 340 postmenopausal women, markers of bone formation (osteocalcin) and resorption (NTx) were assessed in relation to lifestyle factors measured for 2 weeks. [67] Calcium supplementation was associated with 18% decrease in levels of NTx and 8% lower levels of osteocalcin, while alcohol intake was associated with 25% lower NTx levels. The short interval in obtaining the measures of lifestyle variables (2 weeks), the negative bone balance present in postmenopausal women, and the large intra-individual variability and diurnal variation of markers are factors that should be considered when interpreting the results.

Another study showed that women reporting regular alcohol consumption (more than two times per week) showed lowest serum and urinary levels of biomarkers, while men with regular alcohol consumption showed significantly reduced levels ($p<0.05$) of biochemical markers of bone formation (BAP and PICP) and no differences compared to those with moderate alcohol consumption (two times or less per week) for the markers of bone resorption. [68]

Smoking was found to be associated with decreased BMD in both women [69] and men. [70] Current smoking was found to have a negative effect on BMD in females [71] and was associated with statistically significantly reduced levels of bone formation (PICP) markers compared to nonsmokers, but no such pattern was observed in males. [68] However, smoking cessation of six weeks was followed by a significant reduction in NTx marker of bone resorption, which correlated to change in cotinine (a metabolite, byproduct of nicotine) in postmenopausal women ($r = 0.36$, $p < 0.05$), suggesting that changes in bone resorption may be related to smoking. [72]

Difficulty in measuring lifestyle factors over long periods and the fact that changes in bone density are often of the same magnitude as the precision error of measurements even after long periods of time lead to inconsistent results in studies of lifestyle factors and bone loss. [67] As biochemical markers can change between 30-70% during lifestyle changes, and in a shorter interval (within weeks) as compared to BMD measurements, their assessment might offer an alternative to measuring changes in bone mass. [73]

MEASUREMENT OF BONE STRENGTH

Bone densitometry, usually DXA, is the current standard method used in osteoporosis diagnosis. DXA is considered an accurate and precise method. [74,75] However, most DXA methods do not measure true volumetric bone density (g/cm^3), as they measure two-dimensional bone mineral content (g/cm^2) and do not correct for antero-posterior depth. [76] As a result, BMD obtained from DXA is influenced by the size of the area of interest. The term "low bone density" denotes a decrease in bone volume, as used in histomorphometry, but the DXA technique measures an areal mineral density (g/cm^2) rather than a volumetric density (g/cm^3). The term "density" is not always correlated with the true bone density, as in the case of osteomalacia.[18]

The integral measurement of cortical and trabecular components of bone is also an important limitation. [76] Bone mineral density (BMD) measurement at the lumbar spine level presents important inconveniences, such as contour and shape changes due to localized compressions, degenerative changes, and also the presence of aortic calcifications that could falsely elevate lumbar spine BMD measurements. [56,76]

Although dual energy X-ray absorptiometry (DXA) is a repeatable method to study bone mineral density (BMD) with low biological and analytical variation (1%), it is not sensitive to monitor changes in BMD in a follow-up less than one year. The least significant change in BMD measured with DXA is estimated to be 3-4%, with 1-2 years needed between measurements for determining therapy efficacy. [77]

There are also other controversies among experts regarding the criterion for osteoporosis because of difficulty in standardization between instruments and sites. [3] The reported discordance between measurements taken at different sites [78,79] could lead to misdiagnosis if using a single site BMD measurement and generalizing results. [76] It has been observed that BMD measurements at a specific site (e.g. hip, spine) predict fracture at that particular site better than the measurements at other sites. [3]

Bone mass measurements cannot be used for absolute prediction, as no absolute discrimination is possible between patients with fracture and those without, [80] leading to a decreased responsiveness of the tool. In addition, DXA does not include other determinants of skeletal fragility like bone turnover, or its plasticity. [18] Other factors like bone architecture, bone mechanical properties and bone remodeling should also be taken into account when assessing bone strength. [81] The three- dimensional trabecular structure of bone, cortical thickness and cortical porosity may play a role in the likelihood of fracture. [8] There are no methods to measure cortical porosity, but trabecular structure can be investigated using methods like computer tomography (CT) or high-resolution magnetic resonance imaging (MRI). [82]

The radiation exposure in bone densitometry using DXA is very low. However, the radiation exposure for both the patient and the radiographer using the latest models of bone densitometers with a fan beam design is considerably higher, as compared to a pencil beam: 10-60mSv, compared with 1-2.5mSv for DXA [83] (the annual exposure from natural background irradiation has been estimated at 2400 μSv). [80]

Ultrasound measurements are influenced by bone density as well as bone elasticity. Although the exact mechanisms are not fully understood, potential uses include the assessment of bone structure and mechanical properties. [76] Lower costs compared to DXA, portability of equipment and lack of radiation exposure have been cited as advantages of this relatively new measure of bone strength. [8] Although some authors considered the predictive ability of this method to be modest, [84] recent studies showed that quantitative ultrasound (QUS) of the heel predicted hip fracture as well as DXA at the femoral neck, and QUS at the femoral neck provided independent information related to fracture risk than DXA. [3] However, it is uncertain whether the results from clinical trials using DXA can be generalized to patients at risk for fracture identified by QUS. The deficiency in standardization between manufactures and in normative data, as well as moderate correlations with densitometric data limit the introduction of this method in clinical diagnostic practice of osteoporosis. [76]

Quantitative computerized tomography (QCT) was cited as a potential method for osteoporosis diagnosis, but very high radiation exposure and cost limit its broader use. [8] QCT has the ability to differentiate trabecular from cortical bone [85] and measures true volumetric (three-dimensional) BMD independent of bone size.[76] Some authors suggested that discrimination ability between osteoporotic patients and controls is better for CT compared with MRI, probably because of better spatial resolution in CT images. [86]

Magnetic resonance imaging is another potential tool in osteoporosis diagnosis. MRI principles are different than those for the radiographic methods (DXA and CT), which measure the attenuation of the transmitted X-ray after it passes through the body. High magnetic fields are applied in MRI: radio frequency waves produce an excitation of the nuclear magnetic system and generate a signal that is detected in the end.[76] Multiplanar capabilities, three-dimensional character, contrast mechanisms, excellent soft-tissue contrast, and the lack of ionizing radiation have been cited among MRI advantages. [87,88] Also, indications of trabecular architecture, bone metabolism and function have been recently obtained using special MRI techniques (microimaging MRI, spectroscopy, and functional MRI). Measures of trabecular structure like trabecular bone area fraction, trabecular thickness, spacing, and number can be obtained by using high resolution MRI, helping in discriminating between normal and osteoporotic groups.[89] Contraindications for patients, high cost, reduced availability, longer measurement times, and lower spatial resolution than DXA and computed tomography are some disadvantages of MRI. [88,90]

As an alternative to conventional densitometry, which gave no data on bone quality, it has been suggested that bone trabecular structure and quality could be assessed by MRI T1 and T2 relaxation times. [91] A negative correlation between T2 relaxation time and BMD or BMC measured with DXA was noted ($r= -0.40$ and -0.47, respectively). A positive correlation of MRI T1 relaxation time with apparent volumetric BMD also was observed ($r=0.36$, $p<0.05$). [91] As a result, T1 seemed not to be influenced by bone size, and thus constituted a better predictor for true bone mineral density than T2. The age-related substitution of red marrow and bone trabecular structure by fat at the vertebral body level may be the cause for diminished T1 relaxation time. Although these associations proved to be statistically significant, more clinical studies are needed in order to determine if they are useful for clinical purposes.

In vivo and in vitro studies suggest that MRI could provide information on bone composition. Although the mechanisms were not fully elucidated, it has been shown that an increase in fat content accompanies bone loss, and that bone marrow cells are implicated in bone remodeling. [92] In a cadaver study that used excised human vertebrae, low bone signal intensity and long MRI T1 relaxation time were observed when bone mineral was increased or when bone marrow was hypercellular, and increased signal intensity and short T1 relaxation time were noted when an increase in fat compound or histological osteoporotic changes were present.[92] Also, good correlations between T1 relaxation time and BMD measured by single-energy QCT ($r=0.59$, $p<0.0001$), or dual-energy QCT ($r=0.51$, $p<0.0001$) have been found in this experimental study. However, these values need to be tested in a clinical setting to assess if their usefulness for clinical applications.

Methods that do not adjust for antero-posterior depth are subject to variance in results due to differences in bone size. By converting already collected areal BMD to volumetric BMD, the dependence of bone density data on bone size can be eliminated. Although CT provides three-dimensional data (volumetric bone density), its use is constrained by the associated high radiation dose. As the radiographic assessment of spine morphology presents difficulties in accurately and consistency defining anatomical landmarks of spine morphology, [84] MRI using T1-weighted sequence acquisition in the sagital plane could be used to accurately calculate vertebral body dimensions, like vertebral volume and medial sagittal height which could be useful for comparing normal to pathological vertebrae following fractures and monitoring treatment in osteoporosis. [93] MRI was found to be useful in differentiating different types (e.g. metastatic from osteoporotic) and stages of fractures (e.g. recent from old osteoporotic fractures). [93] The authors noted a strong correlation between vertebral body volume and vertebral medial area ($r= 0.95$), with a similar pattern of variation for vertebral bodies' medial areas for each subject (coefficient of variation 5.6%). Kroger et al. (1995) [94] created two MRI measurements at the L3 vertebrae, accounting for bone body volume and antero-posterior diameter. MRI-calculated BMD values correlated with DXA-derived apparent volumetric bone mineral density ($r= 0.66 - 0.84$, $p<0.001$). The authors noted a positive correlation between MRI-calculated vertebral body volume and BMC and areal BMD measured using DXA ($r=0.64$, $p<0.001$ and $r=0.40$, $p<0.05$, respectively), demonstrating that falsely increased BMC and areal BMD values were caused by larger bones. Therefore, normalization of BMD values when comparing data from subjects with different bone sizes was necessary. [94] In a study that compared DXA and MRI methods for assessing femoral neck bone mineral density, Arokoski et al. (2002)[95] observed that although DXA and MRI measurements for volumetric BMD were highly correlated ($r=0.73$, $p<0.001$), DXA measurements lead to lower volumetric bone mineral density and consequently they were less accurate than MRI-obtained values.

It has been suggested that adaptive bone remodeling due to a non-uniform load distribution across the vertebral endplates contribute to vertebral body and endplate alteration and osteophyte growth. [96] In vivo and in vitro studies suggest that MRI could also provide information on vertebral body modifications and structure, [93] including endplate sclerosis and osteophytes in vertebrae that could falsely elevate lumbar spine BMD measurement. [56,76]

Osteophytes are thought to be a bony outgrowth composed of cancelous trabeculae, fatty marrow covered by fibrocartilage, periosteal connective tissue and inflammatory synovium. [97] During osteophyte development, chondrocytic cells in the osteophytes express different types of collagen, along with other cartilage matrix proteins. In the first stage, strong expression of type I collagen is found, while the mature osteophyte has a composition similar to adult cartilage, with abundant presence of collagen type II. [98] Under specific conditions it is possible that chondrocytes differentiate into osteoblasts and their matrix converts from cartilage to osteoid. [97] Therefore, it was suggested that although the stimulus for growth is abnormal, osteophytes may provide a model for studying human bone cell differentiation and the close relationship between chondrocyte and osteoblast differentiation.

The vertebral endplate cartilage at the superior and inferior boundaries of the vertebral body with the disc undergoes calcification with aging and is gradually replaced by bone. [99] Although the prevalence of endplate sclerosis is thought to increase after the age of 50 [100] others suggested that it is not an age-dependent condition. [101] Katz et al. (1988) [102] described endplate sclerosis as abnormal interaction between bone and cartilaginous endplate caused by pathological processes along the vertebral endplate. Endplate calcification also alters the ionic composition around disc cells affecting the metabolism and function of the disc. [103] Therefore, endplate sclerosis has long been thought to play a role in disc degeneration by decreasing nutrient availability to the disc, but this phenomenon is still poorly understood. [99] There is not much known about the biology of the endplate. Endplate cells, similar to annulus fibrous and cartilage produce both type I and type II collagen. [104] Produced matrix proteins like collagen type I, III, VI and X can be altered during aging or the process of degeneration which are difficult to separate. [105] It has been observed that major changes take place in collagen turnover under specific conditions, like in the scoliosis, when a shift in the type of collagen produced in the disc (e.g. more type I, III, VI and IX, X collagen are produced). [105]

As osteophytes cannot be distinguished from bone mineral using DXA, osteophytes were sometimes considered to be an artifact for DXA imaging. [106] Results from studies in women suggested an association between BMD at the spine and osteophytes [106,107] and endplate sclerosis. [108,109,110] Also, osteophytes and endplate sclerosis were found to have a considerable influence on spinal bone mass in elderly women, affecting the diagnostic ability of spinal DXA scans to discriminate osteoporotic women. [108] There are scant data regarding associations of degenerative findings like osteophytes and endplate sclerosis with BMD in men. One study found radiographic features, such as osteophytes and endplate sclerosis to be associated with increased BMD in both men and women and these findings were more frequent in men than women. [111] Also, the study found a trend towards increasing BMD at the spine and hip with increasing grade of osteophyte and endplate sclerosis.

BIOCHEMICAL MARKERS

Bone remodeling is an essential part of bone development. During life, bones are in a continuous evolution encompassing both formation and resorption. After the age of 35-40, the amount of bone formed is less than the bone that is removed, and consequently, bone loss is present. [112] It has been suggested that decreased bone mass and architectural deterioration of bone tissue are related to abnormalities of bone turnover. Besides the changes in bone mass that occur in osteoporosis, histomorphometric studies showed an imbalance between bone resorption and bone formation within a bone remodeling unit, amplified by an overall increase in bone turnover. [77] Skeletal tissue has a monotonous chemical composition: 70% calcium salt (bone mineral), and 30% organic matrix (of which 90% is osteoid formed mainly by type I collagen and 10% non-collagenous proteins), a small number of bone cells (osteoblasts and osteoclasts) and non-collagenous bone proteins. Calcium is deposited or removed depending on formation or degradation of collagen. [113] The biochemical markers are produced as a consequence of bone turnover and they are either proteins or derived products of proteins like enzymes produced by osteoblasts and osteoclasts participating in the bone remodeling or bone matrix constituents. [77,114,115]

Type I collagen, an essential element in the integrity and strength of the bone matrix, is the product of two genes: COLA1 coding for its two α 1(I) chains and COLA2 coding for the α 2(I) chain. [116] Type I collagen presents a triple helix structure, with strands attached by cross-links between lysine or hydroxylysine residues that join the nonhelical amino- and carboxyl-terminal ends of one collagen molecule to the helical portion of an adjacent molecule. [117] Other tissues composed of type I collagen are the skin, dentin, cornea, vessels, fibrocartilage, and tendons. Because collagen turnover is slower in these tissues compared with bone, the skeleton is the most important source of propeptides. [118]

Bone synthesis. Bone formation markers could be either proteins or enzymes released from osteoblasts, like total alkaline phosphatase (ALP), bone specific alkaline phosphatase (BALP) and osteocalcin (OC), or peptides derived from the biosynthetic precursor of type I collagen (type I procollagen), which is secreted by osteoblastic cells. It is 50% larger than the final molecule because of two additional bulky domains at both ends: the amino- terminal or PINP and the carboxyl- terminal or PICP propeptides. These propeptides' role is to prevent the early aggregation of the collagen fibrils inside the cells. Prior to the formation of mature type I collagen molecule, there is a cleavage of the two extension propeptides by two separate enzymes. [113] The propeptides can be detected intact in the interstitial fluid of tissue undergoing rapid collagen synthesis. The use of biochemical markers of bone turnover in assessment of osteoporosis is a relatively new concept and the validation of their clinical usefulness is in its infancy. The first assay for type I procollagen propeptides was that for carboxyl-terminal propeptides (PICP) by Taubman et al. in 1974. [119] This assay was never made generally available. Melko et al. (1990) [120] and Pedersen et al. (1994) [121] described later two similar but more specific assays. The assay elaborated by Risteli and Risteli (1997) [113] that detect PINP in blood, use either the intact form of PINP or a minor form (Col 1-like) as an antigen. This assay showed greater sensitivity than the assay for PICP.

Bone degradation. At both ends of type I collagen, there are two immunogenic parts, known as telopeptides. They serve as the location of intermolecular cross-linking within collagen fibrils. [113] During bone resorption, amino- (NTx) and carboxyl-terminal telopeptides (CTx) of collagen are discharged having cross-links attached. Other markers of bone resorption are: hydroxyproline (Hyp), pyridinoline (Pyr), deoxypyridinoline (dPyr), tartrate-resistant acid phosphatase (TRAP), urinary calcium (Ca) and urinary hydroxylysine glycoside (GHYL).

Some authors consider NTx one of the most accurate markers for bone resorption. [122] NTx is not affected by dietary intake, and therefore no fasting is required. Secondly urine samples are recommended to be used for measuring NTx, and consequent creatinine correction should be performed. [122] The carboxyl-terminal crosslinked telopeptide (ICTP) has been measured in serum, and the assay measures a large fragment of type I collagen in blood that is probably generated by metalloproteinase cleavage of non-mineralized tissue. [113] This is documented by studies on pycnodysostosis, a rare recessive genetic disorder in which patients are devoid of cathepsin K, the proteinase that osteoclasts use primarily to degrade bone collagen during resorption. [123] During the course of this disease, which is useful for understanding the physiology of different telopeptides, very little NTx and CTx are excreted in urine, while ICTP is markedly increased, as ICTP is probably generated by a different proteinase (metalloproteinase). [124] Seemingly ICTP is a poor marker to reflect "physiological" levels of bone resorption by using alternative collagen cleavage sites by other proteases. ICTP seems to be a sensitive marker for pathologically destroyed bone or soft tissues, as in the cases of multiple myeloma, rheumatic arthritis or bone metastases. Thus, it was recommended for use in monitoring pathological degradation caused by pathological conditions like cancer, bone disease or multiple myeloma. [113, 125] As elevated ICTP levels can be used to identify myeloma patients with negative skeletal X-rays, the authors concluded that this biochemical marker could be used in the diagnostic and follow-up process. [125]

In patients with bone marrow edema syndrome (also called transient regional osteoporosis), PINP and ICTP mean serum concentrations were not significantly modified from normal, and therefore no useful information for diagnosis was provided. [126] Yet, due to an increase in bone turnover, the markers' values were elevated in samples obtained from the bone drilling channel. In addition, serum ratios of biochemical markers PINP and ICTP were also increased. These findings along with the lack of osteonecrotic regions could therefore be useful in further understanding of bone marrow edema syndrome.

In contrast with the static measurement of DXA at only one site of the skeleton, the biochemical markers are an indication of the cumulative effect of overall bone metabolism and remodeling in the whole skeleton, which size is considerably influenced by age and gender. [127] They are an indication of the bone activity at the time of measurement. [117] Bone turnover appear to be accelerated in growing children, adolescents and the elderly population. [128] In men, markers of bone turnover reach the highest values in the third decade (20-30 years old). [129] Their levels decrease rapidly in men until the age of 40 years, and between 40 and 60 years remain stable or decrease slightly. [118,130,131] The marker levels are lowest in the fifth and sixth decade and have a small increase in the eighth decade. [129]

Advantages. It is possible to detect changes in bone turnover within 3 to 6 months by using biochemical markers rather than 1-3 years as required for BMD measurements. [6] Although the analytical precision error is higher than that of bone mass measurement: 10-20% as compared to 1% for DXA, the percentage of change under treatment is 40-60% after 3-6 months, while only 3% after 1-2 years with DXA. [77] This could be of clinical value in monitoring responses soon after the initiation of therapy or to predict the risk of fractures. Other advantages include the capacity to detect changes in bone turnover of the entire skeleton, minimal invasiveness, the possibility of repeated evaluation and lack of radiation exposure. [132,133]

Limitations. The variability of the markers is the result of the biologic and analytical (measurement) variability. Factors that affect bone turnover and influence the biological variation are: age, gender, day to day and seasonal variation, immobilization, diet, alcohol, smoking, thyroid and parathyroid dysfunction and hypogonadism. [134] The biological variation is much larger for samples from urine (usually markers for bone resorption) than for serum samples. The day to day variability is very important because it cannot be controlled, and it causes considerable variation, especially to urinary marker measurements.[134,135] During the night bone resorption can reach twice the daytime level.[136] Higher mean levels of bone turnover markers in females, and higher mean serum levels of bone formation markers (except PICP) for males were obtained in winter than in summer.[68] These results lead to the conclusion that bone turnover is accelerated during winter. There is also large variability for each biochemical marker between analytical methods and standards from different laboratories, and a large discrepancy in normal values between laboratories.[134, 137] As urine markers have been reported to vary up to 30% and serum markers by less than 10% within an individual,[138] it has been suggested that multiple measurements should be used to reduce the bone markers intra-individual variation.[112] Bone collagen assay specificity is influenced by the size of cross-links, the possible existence of type I collagen variants, various cross-links in soft tissue collagen, structural variability of the cross-linked telopeptides and the size of the degradation fragments.[113]

Potential uses of bone turnover biomarkers. Most frequently cited potential uses include: early diagnostic, identifying potential slow and fast bone losers as an independent risk factor for osteoporosis, treatment evaluation, increased patient compliance due to feedback and bone disease classification.[6,122]

Most of the prospective studies involving biochemical markers focused on their potential use as *independent risk factors for future fractures*. Akeson et al. (1995)[139] conducted a prospective study in order to assess the value of biochemical markers PICP and ICTP as risk factors for future fractures. The authors found a relationship between PICP and fractures in women 70 to 80 years old, with an odds ratio of 2.4 ($p = 0.036$) for low PICP. For the whole group of women 40-70 years old, the odds ratio for a 1 SD decrease in PICP and subsequent fracture risk was 1.8 ($p = 0.015$), and 1.9 ($p = 0.043$) for ICTP. The authors concluded that the risk for future fracture was associated with decreased PICP and ICTP levels from the mean, and these markers' influence on the risk of future fracture was similar to that of forearm BMC (OR = 1.6, $p = 0.03$).[139] In another prospective study, Garnero et al. (1996)[140] identified type I collagen carboxy-terminal telopeptide (CTx) as a predictive factor for hip fracture, while amino-terminal telopeptide

(NTx) did not predict hip fracture risk in a cohort of more than 7,000 elderly women (the EPIDOS cohort).

An elevated bone turnover in elderly women appears to adversely influence BMD and fracture risk. Ross et al. (2000) [141] found that high bone turnover as assessed by CTx was significantly associated with risk of osteoporotic fracture in a sample of 512 postmenopausal women. In a retrospective study, NTx was found to be positively associated with prior osteoporotic fracture and PICP negatively associated with fracture.[142] Although NTx was the best predictor of age-adjusted BMD when compared to biochemical markers OC, BAP, PICP, Pyd, and Dpd, NTx was not statistically significantly associated with fracture risk and was not an independent predictor of history of osteoporotic fractures. [142]

Ohishi et al. (2000) [143] found that the CTx urinary levels were higher in elderly men who had sustained a hip fracture up to four years earlier as compared to age-matched controls. These results were in concordance with the observation that increased bone turnover is associated with fracture history at all three sites: spine, hip and forearm in women at all ages. [144] Therefore, it was suggested that biochemical markers offer useful information regarding bone metabolism, and that biochemical markers and BMD may provide better prediction of future fracture risk than BMD alone.[77,142]

Several prospective studies related to biochemical markers looked at their potential use to monitor changes in bone formation or resorption levels as a result of *therapy*. Rosen et al. (1997) [145] showed that NTx and serum osteocalcin were the most sensitive and specific predictors of change in spine BMD after a year of hormone replacement therapy in postmenopausal women.

There have been reported significant *correlations between BMD and biochemical markers* like PICP and NTx. PICP was significantly correlated with the initial measure of BMD ($r=-0.64$, $p<0.001$) and showed a significant correlation with a 3 years decrease in BMD in postmenopausal women ($r=0.48$, $p<0.05$). [146] Although in other studies in women markers like PINP and NTx were significantly correlated with rate of BMD loss in the lumbar spine ($r= -0.35$ to -0.52), the prediction of bone loss at the individual level in postmenopausal women was estimated to be poor. [147,148] Bauer et al.(1999) [147] and Garnero et al. (1996) [140] concluded that the use of biochemical markers to predict change in bone density is of limited value for individual, untreated patients after follow-up periods of approximately 4 years.

A longitudinal study in men found a moderate correlation between femoral neck BMD and the change in NTx ($r = -0.26$, $p < 0.05$), [149] while Drake et al. (2003) [150] found no correlation between baseline bone markers (NTx and BAP) and change in femoral neck and spine BMD over a two year period in a group of 77 men 36 to 83 years old. Two longitudinal studies in men reported that PICP and ICTP were not significantly correlated with change in BMD. Scopacasa et al. (2002) [151] found no relationship between the rate of change in forearm bone mineral content and biochemical markers including PICP or ICTP over an interval of 41 months in 123 men 20 to 83 years old. Also, Yoshimura et al. (1999) [152] found that PICP and ICTP were not significantly correlated with change in femoral neck BMD in men 40 to 79 years old, over a 3-year period. As the coefficient of determination for these markers in bone loss prediction was only 5% for lumbar spine and 7% for femoral neck BMD, the authors concluded that these markers cannot predict the change in BMD at the individual level. Other markers of bone turnover, such as urinary CTx and BAP, did not correlate with change in femoral neck BMD, and the correlation with OC ($r=0.31$, $p< 0.01$) did not remain after adjusting for age in a study in men 25 to 86 years old. [153] Similarly, in a previous cross-sectional study, Lormeau et al. (2004) [154] did not find a relationship between biochemical markers and BMD, nor a difference between osteoporotic patients and controls in regards to bone formation (OC and BAP) or bone resorption markers (ICTP and serum CTx). These findings in men seem contradictory to higher correlations between changes in BMD and biochemical markers like PICP and NTx reported in women. [146]

The difference in the results from most studies conducted in men that found weak[155,156] or no significant relationship between markers of bone remodeling and BMD,[157] and those in women that found higher correlations, appear to reflect an influence of gender and hormonal differences. Bone metabolism in men receives lower estrogen influence than in women. Also, the biochemical markers express bone turnover in the whole skeleton, of which size is considerably influenced by gender. [127,158] There are also factors that might add to the overall variation between different study results, like age-related changes, daily variations in individual levels of markers, age-related decrease in glomerular filtration or tubular reabsorption and metabolic rates that are not yet known for biochemical markers. [155]

In conclusion, a mounting number of studies have concluded that changes in biochemical markers cannot replace bone densitometry in the determination of bone mass changes at specific sites of interest, and cannot predict changes in BMD over a period of 3 years. [159,160] To date, most markers of bone turnover available proved to have a limited use in clinical evaluation for individual patients, as they did not show the ability to predict bone loss or fracture risk and were weakly associated with bone mass. However, they have correlated with changes in indices of bone remodeling, and might provide insights into mechanisms of bone loss. [3] The Canadian Consensus Conference on Osteoporosis (2006) [161] noted that bone turnover markers cannot be recommended for prediction of bone loss until more data on clinical applications is available, but they "can be used to rapidly assess adherence to and effectiveness of pharmacological interventions".

THESIS OBJECTIVES

The overall objectives of this thesis were to investigate factors influencing selected bone metabolism marker levels, and the associations of markers with DXA in men. The primary objective of the first study was to examine the relation of body weight, fat and fat-free measures with biochemical markers of bone synthesis (PINP) and degradation (NTx) to gain insights into the mechanisms through which body size and composition influence bone mass among healthy adult men (cross-sectional desingn). The association between marker levels and BMD values at the spine and hip using DXA was also examined (Chapter 2).

The predictive ability of the markers (PINP, ICTP) for BMD changes over a 5-year period was then further assessed (prospective cohort study). The ability of NTx marker in explaing BMD changes over the last 5years was also examined (Chapter 3).

A classic twin study design was used to estimate the role of total heredity (genes and childhood elements), exercise (childhood and adult, leisure time, work-related physical activities, and sports), smoking, calcium intake, weight and other health related factors in determining PINP, ICTP and NTx marker levels (Chapter 4).

Degenerative features based on MRI measurements at the spine were assessed in relation to DXA and markers of bone turnover to investigate whether (1) bone degradation as indicated by decreased BMD will be reflected in the age-related substitution of bone trabecular structure by fat at the vertebral body level indicated by T1 relaxation time, and in an increased endplate concavity; (2) hypertrophic (osteophytes and endplate sclerosis) findings will correlate positively with increased BMD; and (3) the correlation of DXA with bone markers will increase when degenerative findings based on MRI measures are included in the model (Chapter 5).

It should be noted that the samples for the studies described in this thesis came from the 'TwinSpine Study', a research project withing Finnish Twin Study Cohort. The Finnish Twin Cohort contains virtually all Finnish sex-matched twin pairs born before 1958 and alive in 1975.[162] A large cohort of monozygotic and dizygotic twin pairs was drawn from a population-based twin registry, which represents an extraordinary resource for scientific research. Therefore, the population-based Finnish Twin Cohort is likely to be representative for the general Finnish population, as it contains twins originating from all social levels and regions of Finland. Nordic European countries (e.g. Denmark, Finland, Norway and Sweden) have a long tradition of population registration and health-related registers of high quality. [163] The uniquely located registers of large size have been used in representative studies of rare occurrences (e.g the Finnish Twin Cohort consisted in its first phase of over 17,000 like-sexed twin pairs born before 1958).[162]

The Finnish Central Population Registry was used to establish the Finnish Twin Cohort by identifying pairs of persons that were born in the same community, with the same birth date, with the same surname, of same or opposite sex, and alive in 1975. In order to ensure that only biological twin pairs were included in the cohort, an extensive questionnaire was mailed to all the twins in 1975 to obtain data on health related variables (e.g. personal and family history of selected diseases, smoking, alcohol use, weight, height, social support), and to determine zygosity. The response rate was 89%. A second questionnaire was sent in 1981, with a response rate of 84%. Data on hospital usage, cancer incidence and mortality were collected by record-linkage from the Finnish national registry. [162,163]

Monozygotic and dizygotic male twins included in the subject sections of this thesis were selected from the TwinSpine Study who were selected from Finnish Twin Study Cohort, based on discordance in common exposures suspected to influence degenerative musculoskeletal conditions and, in particular, intervertebral disc degeneration and back pain. Bone mineral density and common spine disorders share many of suspected determinants. [3, 70, 162, 164] Co-twins discordant for outcome are an alternative study design for testing epidemiological causal hypotheses, as discordant twins can be considered as matched case-control cases. Data from the two questionnaires sent in 1975 and 1981 were used to select twins that had discordance in occupational materials handling, sedentary work, exercise participation, vehicular vibration and cigarette smoking. Of the pairs who appeared to meet these criteria and were asked if they would be interested in participating in the study, 82% of the pairs volunteered (116 pairs). [162] Thirty-three additional MZ pairs were randomly selected to bring the number of MZ twin pairs to 149. DZ twin pairs (n= 153) were selected in an identical way to that of the MZ twins. Zygosity has been also confirmed by subsequent DNA analyses in these pairs.

A previous analysis regarding representativeness for MZ pairs of the TwinSpine Study as compared to all MZ male pairs from the Finnish Twin Cohort was performed.[165] No significant differences were observed compared to the referents for a level of education, social class, occupational category outdoor vs indoor work, leisure-time physical activity, history of work-incapacitating neck, shoulder or back pain, smoking status, life satisfaction. The only statistically significant differences observed between study pairs and the base population of twins in the Finnish Twin Cohort, were work status and physical loading at work, which is probably due to subjects' selection partly on these characteristics. Subjects were more likely to be working and have higher physical work demands. [165]

Thus, overall the study subjects appear quite representative of the general population from which they were drawn. However, differences in unmeasured factors cannot be ruled out, which could potentially affect the representativeness of the sample and generalizability of the results to the broader population.

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CHAPTER 2: ANTHROPOMETRICS, BIOCHEMICAL MARKERS AND BMD IN MEN

INTRODUCTION

In contrast with dual energy X-ray absorptiometry (DXA) measurements that reflect cumulative effects at only one site in the skeleton, biochemical markers of bone turnover indicate the current systemic activity of bone metabolism and skeletal remodeling. Biochemical markers can detect short-term changes in bone turnover activity, whereas bone mineral density (BMD) changes are detectable only over a period of years.[1,2] This high rate of change in markers in a short period of time is of clinical value in monitoring early responses to various interventions. Other potential uses of biochemical markers cited in the literature are the identification of potential slow and fast bone losers, as an independent risk factor for osteoporosis and future fractures, and for monitoring and predicting changes in BMD. [3,4,5] Biochemical markers have also been used to examine the theory that plasma leptin values may mediate the bone-sparing effects of obesity.[6]

The association between BMD and weight is well established.[7] It has been theorized that weight and muscle mass influence BMD through associated loading [8], and fat through hormonal effects.[9] To our knowledge no one has studied body composition directly in relation to biochemical marker levels. One of the primary objectives of the present study was to examine the relation of body weight, fat and fat-free measures with biochemical markers of bone synthesis and degradation to gain insights into the mechanism through which body size and composition may influence BMD. We theorized that fat-free parameters, such as fat-free body mass index (BMI), could reflect active and passive loading on the skeletal system, leading to decreased bone resorption and consequently higher BMD. Fat-free BMI represents both bone and muscle mass and takes into account height to adjust for bone size and mass. Therefore, it better reflects general muscle mass than fat-free weight or total weight. Also, lean body mass as measured by DXA has been shown to be positively associated with muscle strength, which appeared to have a strong explanatory power for BMD. [10] Thus we examined weight, BMI, percentage of body fat, fat-free weight and fat-free BMI in relation to biochemical marker levels and BMD.

A second objective of the study was to determine the ability of markers to explain the variance in BMD measurements among healthy adult men by assessing the association of biochemical markers with DXA measures of BMD, the current standard method used in osteoporosis diagnosis. An association between biochemical marker levels and BMD has been demonstrated, but few studies have been conducted of men[11,12,13] as compared to women and inconsistencies in findings have led to some controversy. Specifically, we examined levels of serum bone formation marker procollagen aminoterminal propeptide (PINP), urinary amino-terminal telopeptide (NTx), a marker of bone resorption, and the ratio PINP/NTx (as a measure of the imbalance between bone formation and resorbtion) in relation to DXA measurements acquired from a population-based sample of Finnish men. [14]

MATERIAL AND METHODS

A cross-sectional study design was used to examine associations between variables.

Subjects. The current study utilized data available from a larger project, in which twin pairs were selected and recruited from the population-based Finnish Twin Cohort to differ in common exposures suspected of influencing musculoskeletal degeneration (e.g. occupational physical loading). [14] Because collection of data on biochemical markers began after the original "TWIN SPINE STUDY" was underway, the sample size is limited to 173 subjects. The sample for this study was composed of one randomly selected twin from each of 20 monozygotic (MZ) pairs and 153 dizygotic (DZ) pairs with data available for all markers and BMD. Twins ranged in age from 40–70 years (mean 50.2, SD 7.4). Subjects were excluded from analysis if they had a history of the following conditions or medications: hormone (cortisone or steroid) therapy (2); thyroid or parathyroid disorders (5); rheumatic or vasculitis/generalized arthritis (2), hip or spondyloarthritis (2); femur, pelvis or spine fractures, or any fracture **during the prior year** (9); chronic kidney or liver disorders (2); bed rest of more than 1 month during the prior year (5); or cancer in the prior year (2). Subjects with severe degenerative changes in the lumbar spine (e.g. osteophytes) or femoral neck also were excluded from the original study. A total of 28 subjects (16%) were excluded, leaving 145 subjects (130DZ and 15MZ) for inclusion in analyses. A detailed, structured interview including life-style factors like dietary calcium, cigarette smoking, alcohol and coffee consumption also was conducted. In Finland it has been estimated that 74% of dietary calcium comes from milk products[15], and none of the subjects reported taking calcium or vitamin D supplements. Quantity, frequency and beverage type of alcohol consumption were recorded and have been described in earlier papers. [16,17] No statistically significant associations were found between BMD and cigarette smoking, coffee intake and alcohol consumption in an earlier analysis and in the regression analysis dietary calcium explained only 1% of the variance of BMD at the femoral neck and 0% at the lumbar spine.[18] Thus, we did not control for the effects of these factors.

Body composition components. Bioelectrical impedance was used for measuring body fat. The method uses a small electric signal that is circulated while the person is lying down with electrodes are attached to various parts of the body, and it is considered one of the most valid[19] and reliable instruments of estimating body fat (test-retest R= 0.99).[20] Weight was measured using a balance scale, and was recorded to the nearest 0.1 kg. Fat-free BMI was obtained by dividing fat free weight by the square of standing height.

BMD and biochemical markers. Data collection included a structured interview, DXA of the lumbar spine and hip, fasting serum and first void urine samples collected in the morning. Serum and urine specimens were subsequently stored at -20°C to await analysis of PINP and NTx. The assay for PINP was done in Oulu, Finland, in the lab where it was developed (by Dr. Juha Risteli), while the assay for NTx in Seattle, at University of Washington (by Dr. Eyre, the developer). All subjects slept at a hotel adjacent to the testing site the night before the samples were collected. Participants' characteristics, including age, weight, BMD at the various sites, and marker values are summarized in Table 2.1.

PINP is a globular domain at the aminoterminus of the type I procollagen molecule, the biosynthetic precursor of type I collagen. This propeptide can be detected intact in the interstitial fluid of tissue undergoing rapid collagen synthesis. The assays described by Melkko et al. [21] (that detect PINP in blood, use the intact form of PINP or a minor form (Col 1-like) as the antigen. At each end of type I collagen, after removal of the propeptides, short telopeptide domains provide sites of intermolecular cross-linking in collagen fibrils. An assay for cross-linked peptides based on the amino-terminal telopeptide domain (NTx) in urine is in use as an index of bone resorption.[22,23]

PINP was determined from serum by radioimmunoassay using the propeptide as an antigen (Orion Diagnostica, Finland). Intra- and inter-assay coefficients of variation are 4.6-10.3% and 3.1-10.8%, respectively.

NTx, a marker for bone resorption, was measured in urine using an ELISA resorption assay (Osteomark®; Ostex International) and is normalized to urinary creatinine. The biologic intra-individual CV for NTx was found to be 22 % with a range of 16-33%. The analytic intra-assay CV was < 5%, and the analytic inter-assay < 8.0%. NTx values are corrected for creatinine to adjust for the wide-ranging dilution of normal urine spot collections.

Bone mineral density was measured with dual energy X-ray absorptiometry (DXA; Lunar DPX, Madison, WI), at the L1-L4 vertebrae and femoral neck and has been described in detail earlier. [18] The coefficient of variation for BMD measurements was 0.9% for the spine, and 1.5% for the femoral neck. National, ethnic mean values for BMD in 20-29 year old men are 1.06 g/cm² for femoral neck and 1.23 g/cm² for lumbar spine. Standard deviations for BMD are 0.14 g/cm² for the femoral neck and 0.15 g/cm² for lumbar spine. [24] The WHO criteria for white Caucasian women were applied to the national normal database of healthy Finnish men to create limits for normal BMD, osteopenia (BMD between 1 and 2.5 SD below the mean peak gender matched BMD) and osteoporosis (BMD less that 2.5 SD). [25] Using these definitions, three subjects (2%) had osteoporotic lumbar spines (BMD <0.87 g/cm^2) and 39 (26.9%) had osteopenia (0.87-1.08 g/cm^2) at the spine. For the femoral neck, four subjects (2.8 %) were osteoporotic (<0.72 g/cm^2) and 50 (34.5%) had osteopenia (0.72–0.92 g/cm^2).

Data Analysis. Natural logarithms of the marker values were used for NTx, in order that the data would approximate a normal distribution. PINP met normality assumptions without the need of transformation. Pearson's r was used to assess the correlation between anthropometric measures (weight, BMI, percentage of body fat, fat free weight, fat free

BMI), markers and BMD. Also, a multiple partial correlation was performed using the partial correlation coefficient to control for the effects of age, weight and fat free BMI in order to further assess the association of biochemical markers and BMD. A multiple linear regression analysis was conducted to examine the ability of the anthropometric measures mentioned above in explaining NTx and BMD variance, and also the collective ability of the markers to explain the DXA measurements. One-way ANOVA was used to evaluate differences in NTx levels between normal, osteopenic and osteoporotic BMD at the spine and the femoral neck.

The participants received and signed informed consent forms prior to study participation. The study protocols were reviewed and approved by the Ethical Committee of the Department of Public Health at the University of Helsinki, the Human Subjects Committee at the University of Washington, and the Human Research Ethics Board at the University of Alberta.

RESULTS

Weight and other anthropometrics in relation to BMD and biochemical markers

Weight, BMI and fat-free measures were similarly, moderately associated with BMD at the spine (r=0.36-0.41) and femoral neck (r=0.40-0.46). Fat percentage and BMD at either site were not statistically significantly correlated (r=0.05-0.10) (Table 2.2). Height was not statistically significantly correlated with BMD at the spine, and was weakly correlated with BMD at the femoral neck (r=0.2). In multiple regression models, fat percentage did not add to the variance in BMD explained at each site once weight or fat free BMI were included in the model. In the stepwise regression analysis, height was not retained as a predictor for spine or femoral neck BMD.

Similarly, weight, BMI and fat-free measures were more highly associated with NTx (r= -0.25- -0.37) than was fat percentage (r=-0.17). In multiple regression analysis, adding fat percentage did not significantly add to the variance in NTx explained by fat-free BMI.

In multiple regression models fat-free BMI was more strongly associated with spine BMD and NTx than weight. Fat-free BMI explained 16.2% of spine BMD variance, while weight explained no additional variance. Whereas, when weight was forced into the model first it explained 12.1% of BMD variance and fat-free BMI explained an additional

4.8%. Similarly, fat-free BMI explained the highest percentage of NTx variance (11.5%), while adding weight in the model did not increase the variance explained. Again, to examine if fat-free BMI is an independent predictor, weight was entered first and explained 9% of NTx variance, while fat-free BMI explained an additional 3%. In both models, once entered, only the effect of fat-free BMI on NTx was statistical significant ($p<0.05$).

Biochemical marker levels in relation to BMD

Before controlling for age, weight and fat free BMI, NTx was the only marker significantly correlated with BMD at the spine and femoral neck, with Pearson correlation coefficients of -0.26 and -0.29 ($p < 0.01$), respectively. The Pearson partial correlation coefficient for the correlation of NTx with BMD at the femoral neck, after controlling for age and weight and fat free BMI, was -0.20 ($p < 0.05$), and was not significant at the spine (Table 3).

When subjects were separated into groups based on age, NTx was significantly correlated with BMD at the lumbar spine and femoral neck in subjects less than 50 years old ($n = 74$), with Pearson correlation coefficients of -0.26 ($p<0.05$) for the spine and -0.30 ($p<0.05$) for the femoral neck, partialling for age, weight and fat free BMI. However, the corresponding correlation coefficients were -0.03 to -0.09 in those 50 years or older ($p>0.05$). Age was weakly correlated with spine BMD ($r = 0.16$, $p< 0.05$), and PINP ($r= 0.17$, $p<0.05$), but not with NTx when all the subjects were included.

In multiple linear regression models for BMD, NTx explained 6% at the lumbar spine, and 8% at the femoral neck, while fat free BMI added 11%, to the explained variance at each site.

When the participants were divided in three groups based on BMD (normal, osteopenic, osteoporotic) according to WHO recommendations,[21] statistically significant differences in NTx levels were found between osteoporotic and osteopenic subjects ($p=0.006$) as well as between normal and osteoporotic subjects ($p<0.001$) at the femoral neck (Table 4). NTx levels were higher in osteoporotic as compared to osteopenic subjects at the lumbar spine, and in osteopenic compared to normal subjects. The overall difference was statistically significant ($p=0.03$), but not the pair-wise comparisons between groups ($p>0.05$).

DISCUSSION

As stated by the National Institutes of Health (NIH), [26] osteoporosis is not an age or gender-dependent condition. Yet, while there has been a large amount of research focused on women, there is a paucity of information in the scientific literature concerning osteoporosis in men where it also is a frequent condition and serious public health problem.

Weight and other anthropometrics in relation to BMD and biochemical markers

Our findings support the hypothesis that the underlying mechanisms for regulating bone metabolism in men related to anthropometric measures, which in turn enhance BMD, are predominately related to fat-free body composition (representing bone and muscle) rather than fat percentage. In the regression models that considered various anthropometric measures, fat-free BMI accounted for the highest proportion of the variance in NTx and in spine BMD, while body fat did not correlate with BMD at either the spine or hip. Other studies also found that fat-free mass[27] and lean body mass, [8] were the strongest determinants for BMD in men, in contrast to body fat mass measurements, which were not correlated to BMD.

In the present study, fat-free measures representing higher bone and muscle mass were associated with less bone resorption (as estimated by NTx) and higher BMD values. Because the fat-free measurements could not be separated into muscle and bone, we can only speculate about the underlying mechanisms for the associations with fat-free measures. The finding that fat-free measures were more strongly and independently associated with BMD and NTx than weight suggests that these relationships are not explicable simply in terms of weight-bearing effects alone, but may be partly due to greater physical activity and associated physical loading. The higher association of fat-free measures with BMD and biochemical markers in men than in women is consistent with this view.[8,27] However, although bone mass accounts for only 4-8% of fat-free mass variance, [28] we can not rule out the possibility that the association between low NTx level and high BMD is through other mechanisms than physical loading due to body weight and muscle force. The association could be explained by other determinants of bone metabolism affecting both bone and NTx levels.

Among women body fat was the most significant determinant of BMD.[6,8,9] It has been suggested that this is due to leptin, a hormone secreted by adipose cells and osteoblasts that was recently found to be positively associated with BMD in women.[9] However, Goulding and Taylor found no association between circulating plasma levels of leptin and markers of osteoblastic or osteoclastic activity in postmenopausal women, suggesting that leptin does not mediate the effects of obesity on BMD in that group of subjects.[6] The gender difference in the association between fat mass and BMD may be explained by genetic determination of muscle mass in association with bone growth, higher testosterone levels, or more intensive physical activity among men.[8] Unfortunately, we did not have data on estrogen, testosterone and other hormones with possible influences on metabolism and muscle strength, and it would be important to see the relation of these hormones with BMD and the biochemical markers in both males and females to further enhance our understanding of mechanisms implicated in bone turnover. Also, bioelectrical impedance used in this study may be a less accurate and reproducible method than DXA for measuring body fat and muscle, which would have the effect of diluting the apparent role fat free BMI due to measurement error.

Biochemical marker levels in relation to BMD

Of the biochemical markers studied, only urinary NTx was negatively correlated with BMD, before controlling for age, weight and fat free BMI in men from 40 to 70 years of age. Similar to the present study, in a study on healthy Japanese men 48-85 years old, Iwamoto et al. [29] found an inverse correlation between NTx and metacarpal BMD ($r= -0.33$, $p<0.01$), suggesting an increased bone resorption. The greater association between NTx and BMD, as compared to PINP may be, in part, because NTx is more specific to bone type I collagen resorption by osteoclasts than PINP is to bone type I collagen formation by osteoblasts. This is in agreement with an earlier study in men using serum NTx and PINP. [30] However, in multivariable analysis NTx accounted only for 6-8% of the variance in BMD at the femoral neck and spine, while fat free BMI added 11% to the explained variance. These modest relations are not unexpected considering that type I collagen is not bone specific and is found throughout the body. [31] Moreover, there are daily variations in individual levels of markers affecting measurement values, and they reflect turnover within the whole skeleton, rather than that of a specific site (as viewed by

DXA). In addition, the bone markers reflect current bone activity [32] while DXA reflects the lifetime cumulative outcome of bone metabolism, including the peak bone mass reached in early adulthood.

Earlier studies reported bone loss in men after the age of 50. [33,34] Therefore, the correlations NTx with BMD were also studied among subjects younger and older than 50 years. Surprisingly, we found higher correlations of NTx to BMD (r= -0.26 for the spine, and -0.30 for the femoral neck) in subjects from 40 to 50 years old than in subjects from 50 to 70 years old (0.03 to -0.09), where correlations were not statistically significant. The results suggest the presence of bone resorption in the 40-50 year age group, associated with less BMD. To our knowledge, this is the first cross-sectional study on the correlation of urinary NTx with BMD that includes healthy middle-age adult men under 50 years old.

Lower correlations in the older subjects in the present study could be due to bone remodeling such as osteophyte formation, calcifications, sclerotic changes in the vertebrae, or decreased renal function which happen in later life, that could mask decreased BMD in the trabecular bone. The lack of an association between NTx and BMD at the spine in men over 50 years old is consistent with this possible explanation and the results of other studies.[11,12] However, contrary to our findings is the inverse association between NTx and BMD that has been found in older men at the femoral neck.[11,12] It might be possible that besides the limited sample of osteoporotic men, hip osteoarthritis and limited physical activity in the elderly group would affect the results. Also, in the studies of elderly men over 70 years old, the current rate of bone loss might be higher than in this sample of men 50-70 years old, and markers of bone resorption such as NTx may better reflect BMD, as the decrease in BMD might be accentuated. The role of elevated parathyroid hormone or low testosterone levels in older men should also be addressed by future studies in order to better understand their effect on BMD and biochemical markers of bone turnover, and the variations between age groups.

Furthermore, the ability of NTx levels to differentiate between BMD categories in this population-based, healthy cohort of adult men was limited by the small number of osteoporotic men (only 3 at the spine and 4 at the femoral neck from a total of 145 subjects). NTx levels were different between men with normal and osteoporotic BMD levels at the femoral neck, but not at the spine. These results are similar to those obtained by Schneider el al.,[11] where NTx discriminated between normal, osteopenic and osteoporotic BMD levels only at the hip but not in the spine in men.

CONCLUSIONS

Of the anthropometric variables studied, fat-free BMI was the strongest determinant for NTx, PINP and spine BMD, supporting our hypothesis that fat-free parameters reflecting bone and muscle mass play a more important role in bone metabolism than body fat in men. The underlying mechanism of the fat-free BMI to enhance BMD is related to decreased resorption as assessed by the NTx marker. Urinary NTx was a better indicator of current BMD status than serum PINP, and NTx levels were significantly different between men with normal and osteoporotic BMD at the femoral neck.

Table 2.1. Summary characteristics for participants

VARIABLE	N	MEAN (SD)	RANGE
AGE (YEARS)	145	50.1 (7.3)	40 - 70
HEIGHT (CM)	130	175.2 (5.4)	160 -190
WEIGHT (KG)	145	79 (11.9)	54 - 120
BODY FAT (%)	140	20.8 (4.5)	10.7 - 33.7
FAT-FREE MASS (KG)	140	62.6 (8.1)	46.6 - 88.8
FAT-FREE BMI (KG/CM^2)	127	20.3 (2.2)	14.5 - 26.5
SPINE BMD (G/CM^2)	145	1.2 (0.2)	0.8 - 1.8
FEMUR NECK BMD (G/CM^2)	145	1.0 (0.1)	0. 7 - 1.3
PINP (μG/L)	145	36.5 (13.7)	11.2 - 71.5
NTX (nM BCE/ mM CREATININE)	145	31.9 (15.7)	12.6- 110.6
PINP/NTX	145	1.3 (0.5)	0.2 - 2.8

Table 2.2. Pearson Correlation Coefficients for anthropometric factors and BMD and markers of bone turnover

	BMD SPINE	BMD FEMORAL NECK	PINP	NTX	PINP/NTX
WEIGHT	0.36^{b}	0.46^{b}	-0.04	-0.29^{b}	0.26^{b}
FAT %	0.05	0.10	0.03	- 0.17^{a}	0.21^{a}
BMI	0.36^{b}	0.40^{b}	-0.18^{a}	-0.37^{b}	0.20^{b}
FAT-FREE WEIGHT	0.40^{b}	0.46^{b}	-0.03	-0.25^{b}	0.22^{a}
FAT-FREE BMI	0.41^{b}	0.42^{b}	-0.21^{a}	-0.35^{b}	0.15
HEIGHT	0.098	0.18^{a}	0.28^{b}	0.03	0.20^{a}

^{a} $p < 0.05$, ^{b} $p < 0.01$

Table 2.3. Pearson Partial Correlation Coefficients[a] for BMD at the lumbar spine and femoral neck, and markers of bone turnover (n=145)

	PINP	NTX	PINP/NTX
SPINE BMD	-0.05	-0.12	0.05
FEMURAL NECK BMD	-0.05	-0.20[b]	0.14

[a] adjusted for age, weight and fat free BMI
[b] $p < 0.05$

Table 2.4. Urinary N-Telopeptide (NTx) levels by WHO Diagnostic Criteria[a] at the lumbar spine and femoral neck

WHO DIAGNOSTIC CRITERIA	LUMBAR SPINE NTX MEAN (SD)	FEMORAL NECK[b] NTX MEAN (SD)
NORMAL, <-1.0 SD	N= 103 3.31 (0.42)	N=91 3.29 (0.41)
OSTEOPENIA, > -1.0 and < -2.5 SDs	N=39 3.48 (0.42)	N=50 3.44 (0.38)
OSTEOPOROSIS, > -2.5 SDs	N=3 3.73 (0.07)	N=4 4.12 (0.21)

[a] WHO recommends diagnostic criteria based on comparing bone mineral density with gender-specific mean peak bone mass of the reference data. NTx was calculated from log-transformed data.

[b] Statistically significant differences ($p<0.05$) in NTx levels between normal-osteoporotic and between osteopenic-osteoporotic subjects were found at the femoral neck.

In the spine, the overall comparison was significant, but not the individual contrasts.

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CHAPTER 3: THE PREDICTIVE ROLE OF BIOCHEMICAL MARKERS FOR CHANGE IN BMD IN MEN

INTRODUCTION

The use of biochemical markers of bone turnover, as indicators of overall bone metabolism, has been suggested as a potentially valuable clinical method in osteoporosis for screening, diagnosis, and monitoring the effects of different interventions. [1] Apart from fractures, dual-energy x-ray absorptiometry (DXA) is the only currently accepted method of diagnosis for osteoporosis. It has been suggested that decreased bone mass and architectural deterioration of bone tissue are related to abnormalities of bone turnover[2]. Biochemical markers reflect small changes in bone turnover of the entire skeleton and in a shorter time frame compared to the months or years that it can take to visualize distinct changes in bone mineral density (BMD) using absorptiometry methods. [2] They might also capture bone properties independent of BMD measurements, like bone remodeling, fragility, trabecular connectivity or architecture. [3]

As there is a great need to identify persons at risk for osteoporosis, it would be useful to obtain a quantitative evaluation of rates of bone loss to identify "fast bone losers" in an early stage [4] in order to target interventions to decrease further rapid loss of bone. Population studies showed that biochemical measurements may predict rates of bone loss and occurrence of fractures. [4] An elevated bone turnover in elderly women appears to adversely influence bone mass and fracture risk. It has been suggested that combined biochemical markers and BMD screening might better predict future fractures than BMD alone. [5] Studies in which biochemical markers predicted osteoporotic fractures independent of BMD suggested that the measurement of BMD and the biochemical assessment of bone turnover may be complementary in prediction of bone strength and possible future fractures. [6,7] Understanding the complex interrelationship between BMD and bone turnover markers could facilitate the creation of better predictive models and interventions for osteoporosis that include these factors. Although osteoporosis in men is a serious and frequent condition, there is a paucity of information in the scientific literature concerning osteoporosis in men.

The aim of this study was to determine whether specific markers of bone formation, including type I procollagen amino- terminal propeptide (PINP) and type I procollagen carboxyl- terminal propeptide (PICP), and of bone resorption, type I collagen carboxyl-terminal telopeptide (ICTP), are predictive of changes in BMD at the lumbar spine and femoral neck over a 5-year period in a group of men 35–70 years old. PINP and PICP are globular domains at the amino- terminus and carboxyl-terminus, respectively, of the type I procollagen molecule, the biosynthetic precursor of type I collagen fibrils. The role of these propeptides is in monomer formation, preventing early aggregation inside the cells and aiding ordered fibrillogenesis. The propeptides can be detected intact in the interstitial fluid of tissue undergoing rapid collagen synthesis and in serum. At each end of type I collagen, after removal of the propeptides, short telopeptide domains provide sites of intermolecular cross-linking in collagen fibrils. An assay for cross-linked peptides based on the amino-terminal telopeptide domain (NTx) is in use as an index of bone resorption. [8] A second objective of the present study was to determine the ability of urinary bone resorption marker amino-terminal telopeptide (NTx) to explain the variance in BMD change over the past 5 years.

The hypothesis was that the correlations of markers with either previous or future BMD changes (e.g. either in the prior or subsequent 5 years) would be higher than those found in a previous cross-sectional analysis, including some of the same subjects. [9] The markers, which represent bone turnover activity were expected to better reflect relatively recent changes in bone mass than a measure taken at one point in time reflecting cumulative lifetime influences on BMD.

MATERIAL AND METHODS

Both prospective and retrospective cohort study designs were used to examine the ability of bone turnover markers to predict change in BMD. The biochemical markers PINP, PICP and ICTP were measured on samples collected in 1992, and urine NTx on samples collected in 1997. DXA data were gathered in 1992 and again in 1997.

Subjects. The current study utilized data available from a larger project, in which twin pairs were selected and recruited from the Finnish Twin Cohort, which is representative of the Finnish population. The Finnish Twin Cohort contains virtually all Finnish sex-matched twin pairs born before 1958. The twin pairs were selected solely on within pair discordance for a common exposure suspected of influencing musculoskeletal degeneration (e.g. occupational physical loading). [10]

Earlier analyses found the selected subjects to be quite representative of the Cohort from which they were drawn on a number of factors investigated. Twins originate from all social levels and all regions of Finland. No significant differences were observed compared to the referents for level of education, social class, occupational category, outdoor vs. indoor work, leisure-time physical activity, history of work-incapacitating neck, shoulder or back pain, smoking status and life satisfaction. The only statistically significant differences observed between study pairs and the base population of twins in the Finnish Twin Cohort were work status and physical loading at work, which is probably due to subjects' selection partly on these characteristics. Subjects were slightly more likely to be working and to have higher physical work demands. [10]

Collection of data on biochemical markers began after the original study was underway, thus the sample size is limited to 240 subjects 35-70 years old. The study sample for this study was composed of monozygotic (MZ) pairs with data available for all markers and BMD. The participants provided informed consent forms prior to study participation. The study protocols were reviewed and approved by the Ethical Committee of the Department of Public Health at the University of Helsinki and the Human Research Ethics Board at the University of Alberta.

A detailed, structured interview including lifestyle factors like dietary calcium intake, cigarette smoking, alcohol and coffee consumption also was conducted. In Finland it has been estimated that 74% of dietary calcium comes from milk products, [11] and none of the subjects reported taking calcium or vitamin D supplements. Quantity, frequency and beverage type of alcohol consumption were recorded and methods have been described in earlier papers. [12, 13] No statistically significant associations were found between BMD and cigarette smoking, coffee intake and alcohol consumption in an earlier analysis and dietary calcium explained only 1% of the variance of BMD at the femoral neck and 0% at the lumbar spine. [14] Thus, we did not control for the effects of these factors in the present study. Subjects were excluded from analysis if they suffered any chronic kidney or liver disorders (3), or if they had a history of the following conditions or medications in the prior year: thyroid or parathyroid disorders (0); hormone (cortisone or steroid) therapy (4); epilepsy or anti epilepsy medication (3); any skeletal disease or fracture (21); bed rest of more than 1 month (3); or active cancer (3). Thus, a total of 37 subjects were excluded, leaving 203 (82%) subjects with cumulative marker and BMD data for inclusion in analyses. The twins ranged in age from 35 – 69 years at baseline (mean 49.7, SD 8.4). Additional characteristics of participants are provided in Table 1.

BMD and biochemical markers. Data collection included DXA of the lumbar spine and right hip collected in 1992 and 1997, fasting serum and first void urine samples collected in the morning. Serum and urine specimens were subsequently stored at –20°C at the study site hospital. The samples were then moved to another laboratory and stored at -70°C to await analysis of the markers. Serum specimens for PINP, PICP, and ICTP were collected in 1992, while urine specimens (NTx) in 1997. All subjects slept at a hotel adjacent to the testing site the night before the samples were collected.

PINP was determined in serum by radioimmunoassay using the propeptide as an antigen (Orion Diagnostica, Finland). The reference interval in men ranges from 20-76 µg/l. Intra- and inter-assay coefficients of variation (CV) are 4.6-10.3% and 3.1-10.8%, respectively. PICP serum concentrations were analyzed with radioimmunoassay kits (Orion Diagnostica, Finland). The intra and inter-assay CV were < 6%.

NTx was measured in urine using a competition ELISA assay (Osteomark®; Ostex International) and is normalized to urinary creatinine. NTx values are corrected for creatinine to adjust for the wide-ranging dilution of normal urine spot collections. The biologic intra-individual coefficient of variation (CV) for NTx was found to be 22 % with a range of 16-33%. The analytic intra-assay CV was < 5%, and the analytic inter-assay < 8.0%.

ICTP, another marker of collagen type I degradation, was analyzed in serum with a radioimmunoassay using polyclonal antibodies against the C-telopeptide region of type I collagen, which were produced in rabbits (Orion Diagnostica, Finland). The reference interval in men ranges from 1.6- 4.6 μg/l. Intra- and inter-assay coefficients of variation are 2.8-6.2% and 4.1-7.9%, respectively.

Bone mineral density was measured with dual energy X-ray absorptiometry (DXA; Lunar DPX, Madison, WI), at the L1-L4 vertebrae and right femoral neck and has been described in detail earlier[14]. The coefficient of variation for BMD measurements was 0.9% for the spine and 1.5% for the femoral neck. National, ethnic mean values for BMD in 20-29 year old men are 1.06 g/cm² for femoral neck and 1.23 g/cm² for lumbar spine. Standard deviations for BMD are 0.14 g/cm² for the femoral neck and 0.15 g/cm² for lumbar spine. [15] The same type of DXA equipment and software for pencil beam densitometers (DPX) were used in the present study at baseline and follow-up.

Data Analysis. The STATA statistical package was used for data analyses. The α level was set at 0.05 for determining statistical significance. Participants' characteristics, including age, change in BMD at the various sites, and marker values are summarized in Table 1. Because the use of twin pairs violates the assumption of independence required for standard statistical models, except when noted, all standard errors were adjusted for clustering by twinship. The intraclass correlations between the twins for our outcome variables range from 0.17 for ICTP to 0.50 for NTx. This reduces our effective sample size from 126 to 84-108. However, as the minimum sample size calculated was 60 ($n = L/f^2 + k + 1$, where $f^2 = R^2/1-R^2$, k = number of variables = 2, R^2 = variance to be declared significant and therefore $n = L/0.25 + 2 + 1 = 14.17/0.25 + 2 + 1= 56.68 + 2 + 1 = 60$), even 84 is a reasonable sample size, with > 80% power to detect effect sizes as low as 0.31 of one standard deviation for the outcomes.

Pearson coefficients assessed the correlation between spine and femoral neck BMD and marker values. Multiple linear regression analyses were conducted to examine the ability of markers to explain change in BMD, with age, fat-free weight, height and baseline BMD introduced in the model as possible confounding factors. The adjusted R^2 (AR^2) indicated the percent of the variance explained by a covariate. Finally, the effect of familial aggregation or twinship on an outcome was determined by entering indicator variables for each twin pair (save one) into the model, and examining the AR^2. No adjustment of the standard errors was necessary in those models. One-way ANOVA was used to evaluate differences in NTx levels between the 15% of subjects with the greatest changes in BMD (a gain or loss) vs. the remainder of the subjects with lesser degrees of change in BMD at the lumbar spine and femoral neck.

RESULTS

Among the markers studied, NTx, a marker of bone resorption, measured at the follow-up correlated with the change in femoral neck BMD during the previous 5 years ($r= -0.21$, $p= 0.006$). NTx explained 3.8% of the change in femoral neck BMD. The other variables (age, fat free weight, height) did not significantly add to the variance explained in femoral neck BMD, with the exception of baseline femoral neck BMD, which brought the total explained variance to 6.7%. Higher NTx and baseline femoral neck BMD were associated with greater decreases in BMD. Familial aggregation did not significantly add to the explained variance. In the cross-sectional analysis NTx correlated only with BMD at the femoral neck ($r= -0.3$, $p= 0.001$), and explained 8% of the variance ($p=0.001$). There was no statistically significant difference found in NTx levels between subjects with the greatest BMD changes and subjects with lesser changes ($p= 0.2$).

Baseline levels of bone formation markers PINP and PICP, and of the bone resorption marker ICTP, did not significantly correlate with the change in spine or femoral neck BMD in the subsequent five years (Table 2). Although non-significant ($p= 0.06$), PINP levels explained 3.4% of the variance in spine BMD change when introduced in the regression model. Age explained an additional 5.9% of the variance. None of the other variables (fat-free weight, height and spine BMD at baseline) was remotely significant. When added to the model, familial aggregation explained 23% of the variance.

Markers of bone formation and bone degradation were interrelated. PINP and PICP markers of bone formation measured at baseline correlated with NTx measured at follow-up ($r= 0.36$, $p<0.01$, and $r= 0.2$, $p= 0.03$ respectively). ICTP measured at baseline correlated with NTx measured at follow-up ($r= 0.2$, $p= 0.04$).

DISCUSSION

The study was conducted over a period of 5 years, and as far as we know it is the longest longitudinal study on biochemical markers of bone turnover in relation to change in BMD conducted in adult men. Among the biochemical markers investigated, NTx was the only marker to correlate significantly with changes in BMD during the previous 5 years at the femoral neck, but not at the spine. The markers of bone formation, PINP and PICP, and the marker of bone resorption, ICTP, did not correlate significantly with change in spine or femoral neck BMD.

In men, markers levels tend to be highest in the third decade (20-30 years old) corresponding to formation of peak bone mass, decrease rapidly from the third decade until the age of 40 years, and remain stable or decrease slightly between 40 and 60 years. [16-18] Marker levels are lowest in the fifth and sixth decade, with a increase in bone resorption markers in the eighth decade. [18,19]

Given this and the age of the subjects in the study (35-69 years), a small variation in the marker levels and the bone loss over this age range might be a factor limiting the associations. However, in this study group, NTx values had a wide range (min= 13, max=132, mean= 39, SD= 16), as did the values for change in BMD (min= -0.21, max= 0.16, mean= 0.01, SD= 0.05). Yet, even when comparing the mean NTx in the subjects with the greatest changes in BMD vs. those with lesser changes, there was no statistically significant difference in NTx levels between these groups. The small number of subjects older than 60 years (N= 15) limits our ability to extend the results of this study to prediction in the elderly.

NTx, a marker of bone resorption measured at follow-up correlated with the change in BMD at the femoral neck during the previous 5 years ($r = -0.21$, $p<0.05$), similar to earlier cross-sectional findings using subjects selected from the same data base, demonstrating that among the markers studied only NTx correlated with BMD at the femoral neck ($r= -0.3$, $p<0.05$). [9] In the present study NTx explained only 3.8% of the variance in change in femoral neck BMD, while in both previous [9] and current cross-sectional analyses, NTx explained 8% of the variance. Another longitudinal study in healthy, elderly ambulatory male volunteers 76 years and older found a similar correlation between femoral neck BMD and the change in NTx ($r = -0.26$, $p < 0.05$), [20] while no correlation between baseline bone markers (NTx and BAP) and change in femoral neck and spine BMD over a 2-year period was found in a group of osteoporotic men 36 to 83 years old. [21] Other markers of bone turnover, such as bone resorption markers (ICTP and serum CTx), osteocalcin (OC) and bone alkaline phosphatase (BAP) did not correlate with change in BMD in studies in men 25 - 86 years old [22] and 42 - 65 years old respectively.[23]

Although nonsignificant, the low correlations of PINP with change in BMD at the spine and hip approached significance, as did the correlation of PICP with change in spine BMD. Other longitudinal studies in men of shorter duration also have reported that PICP and ICTP were not significantly correlated with change in BMD. Scopacasa et al. (2002)[24] found no relationship between the rate of change in forearm bone mineral content (BMC) and biochemical markers including PICP or ICTP over an interval of 41 months in 123 healthy men 20 to 83 years old. Also, Yoshimura et al. (1999) [25] found that PICP and ICTP were not significantly correlated with change in femoral neck BMD in a cohort of Japanese men 40 to 79 year-old residents in the same town, over a 3-year period. As the coefficient of determination for these markers in bone loss prediction was only 5% for lumbar spine and 7% for femoral neck BMD, the authors concluded that these markers cannot predict the change in BMD at the individual level. Thus, our results confirm the findings of other studies conducted in adult men and suggest that the use of current biochemical markers to predict change in bone density in the general male population is of little value.

A limitation of longitudinal studies using DXA measurements is that the measurement error in such follow-up studies can be expected to be greater relative to the magnitude of the BMD measure of change than for a single measure of BMD in a cross-sectional study. In the present study differences between baseline and follow-up DXA measurements due to methodological variations should be minimal, as the same type of DXA equipment (GE Lunar corp. Madison, WI) and software for pencil beam densitometers (DPX) were used at baseline and follow-up. A possible explanation for the lack of clear association between the markers and BMD change could be that the change in BMD over 5 years was of insufficient magnitude to overcome the dilution of correlations by measurement error in the markers and BMD measurements. The present study and most others also are limited by the absence of measures and lack of control of factors, such as insulin-like growth factor-1 or parathyroid hormone, which have been found to correlate with a decline in BMD[22] and the sex-hormone binding globulin, which was associated with hip and spine BMD and CTx marker levels. [23] In any event, in middle aged men, PINP, PICP and ICTP were not informative of future change in BMD over a five-year period.

Another possible explanation for the low association between the markers and BMD in our study and others of men could be that biochemical markers are connected to aspects of bone quality rather than to bone mass. They might capture more dynamic bone properties like bone remodeling or architectural characteristics independent of BMD measurements. Therefore, they could be a contributing factor in risk of fracture even if bone mass measurements are unchanged or do not predict change in BMD.

In contrast to men, higher correlations between changes in BMD and biochemical markers like PICP, PINP and NTx have been reported in women. [26, 27] However, Bauer et al. (1999) [26] and Garnero et al. (1996) [27] suggested that the use of the available biochemical markers to predict change in bone density is of limited value for individual, untreated elderly women.

The difference in the results from most studies conducted in men, which found weak [28, 29] or no significant relationship between markers of bone remodeling and BMD, [16] and those in women which found higher correlations, appear to reflect an influence of gender and hormonal differences. The results of several studies suggest that there are factors related to menopause that could contribute to a greater variation in bone metabolism in postmenopausal women when compared to premenopausal women or men[30, 31]. The biochemical markers express bone turnover in the whole skeleton, of which size is considerably influenced by gender. [32, 33] Also, bone metabolism in men is less affected by the influence of estrogen than in women. Therefore, changes in bone mass in women might be of greater magnitude that those found in men, which might not be detectable through statistical methods as they might also be comparable in size to the measurement error. There are also factors that might add to overall variation between different study results, like age-related changes, daily variations in individual levels of markers, age-related decrease in glomerular filtration or tubular reabsorption and metabolic rates that are not yet known for biochemical markers. [18] However, we cannot exclude that a higher rate of bone loss might have occurred in the last period of the 5 years of follow-up. Therefore, a measurement of bone turnover (e.g. NTx marker) that taken in the same period of time with the second BMD measurement might be a better reflection of recent changes in BMD than a measurement of bone turnover taken 5 years earlier (e.g. PINP, PICP and ICTP markers).

CONCLUSIONS

The correlations of markers with previous or future BMD changes (over a 5-year period) were not higher than those found in a cross-sectional analysis in men 35 to 69 years old. Baseline PINP, PICP and ICTP marker levels did not predict change in spine and femoral neck BMD in this group, and NTx levels explained a statistically significant yet quite limited portion of the variance in change in femoral BMD over the prior five years. Thus, we conclude that the usefulness of these markers in predicting age-related change in BMD is of limited value in adult men.

Table 3.1. Summary characteristics of participants

Variable	N	Mean (SD)	Min	Max
Age (years)	197	49.7(8.4)	35	69
5-year change in spine BMD (g/cm²)	127	-0.02(0.05)	-0.21	0.11
5- year change in femoral neck BMD (g/cm²)	128	- 0.001(0.05)	-0.19	0.16
PINP(μg/l)	200	46.8(15.7)	16	132
PICP(μg/l)	200	141(42)	66	354
ICTP(μg/l)	201	2.9(0.9)	2	8
NTX (nM BCE/mM creatinine)	128	39.3(16.6)	13	132

Table 3.2. Pearson Correlation Coefficients for baseline levels of markers of bone turnover and subsequent change in BMD

BMD	PINP[1]	PICP[1]	ICTP[1]	NTx[2]
Change in spine BMD	0 .20	0.20	0.05	0.09
N	126	126	127	127
Change in femoral neck BMD	-0.13	-0.06	-0.01	-0.21(*)
N	127	127	128	128

*Correlation is significant at the 0.05 level (2-tailed)
1 = measurements at baseline
2 = measurements at follow-up
N= number of observations

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CHAPTER 4: GENETIC AND ENVIRONMENTAL INFLUENCES ON BONE TURNOVER MARKERS – A STUDY OF MALE TWIN PAIRS

INTRODUCTION

The deterioration of bone tissue is considered to be related to an imbalance between bone formation and bone resorption and may be captured by biochemical markers. While use of biochemical markers as indicators for different aspects of bone remodelling has been more extensively described in women, osteoporosis is also a health issue for men. Markers provide an indication of overall skeletal remodelling over short intervals (within weeks), as compared to DXA which is not sensitive to monitor changes in bone in a follow-up less than one year. [1] Therefore, their assessment might offer additional or alternative information in measurements of bone. In general, increased bone turnover rates assessed by markers have been associated with decreased bone mass in women, [2,3] but relatively scant data on biochemical markers is available in men. [4-6]

Biochemical markers of bone remodeling represent enzymes or proteins secreted by bone cells, byproducts that are produced during bone synthesis, or breakdown products from bone resorption. [7] The markers that originate from type I procollagen or collagen are thought to be relatively specific for bone, as the skeleton is considered to be the most important source of type I collagen. [8] Other tissues like the skin, dentin, cornea, vessels, fibrocartilage, and tendons also contain type I collagen, but collagen turnover is slower in these tissues as compared to the bone.

Type I collagen, the most abundant protein in the human body is an essential element in the integrity and strength of the bone matrix and is the product of two genes: COLA1, the gene coding for its two α1 (I) chains and COLA2, the gene coding for the α2 (I) chain. [9] Type I procollagen, the biosynthetic precursor of type I collagen, is secreted by osteoblastic cells and is 50% larger than the final molecule because of two additional bulky domains at both ends: the amino- terminal or PINP and the carboxyl- terminal or PICP procolagen type I propeptides. Prior to the formation of the mature type I collagen molecule, the two extension propeptides are cleaved from procollagen type I by two separate enzymes and are found in the interstitial fluid of tissue undergoing rapid collagen synthesis. [10] At both ends of type I collagen there are two immunogenic parts, known as telopeptides that serve as the location of intermolecular cross-linking within collagen fibrils [10] and are cleaved during the process of collagen degradation. In order to assess the bone degradation level, amino-terminal telopeptide (NTx) has been measured in urine, and the carboxyl-terminal crosslinked telopeptide of type I collagen (ICTP) has been measured in serum. The telopeptides are not affected by diet, but the markers measured in urine, such as NTx need to be normalized to creatinine in order to adjust for the wide-ranging dilution of normal urine spot collections. [11,12]

The majority of studies on biochemical markers focus mainly on clinical applications and do not consider the markers as a central part of their evaluation. [7] Previous twin studies indicated that genetic factors are influencing the markers and that bone turnover is heritable. However, these studies focused exclusively on women, especially postmenopausal twins. [13-16]

Also, physical activity and nutritional factors such as calcium, phosphate, vitamin D and protein intake are thought to be factors that might influence bone turnover and marker levels. [17] Difficulties in reliably measuring behavioral and environmental factors over long periods, are likely responsible for the inconsistent results found in studies of such factors and bone loss. [18] Many limitations of epidemiological, especially case-control studies can be overcome by the study of twins, as twins are uniquely matched for age, sex and multiple confounding variables. Therefore twin studies enable a powerful design using a relatively small sample.

The present study is the first to examine in men genetic, other constitutional and behavioural factors that could influence recently elaborated markers of bone formation or bone resorption that originate from type I procollagen or collagen using a classic twin study design based on monozygotic (MZ) and dizygotic (DZ) twins. The aim of the study is to estimate the role of overall inherited influences (both genes and shared family childhood elements), as well as other suspected influential factors, such as body weight, lifetime physical activities at work and leisure time, smoking, alcohol, calcium intake and other health related factors in determining procollagen type I amino-terminal propeptide (PINP), type I collagen carboxyl-terminal telopeptide (ICTP) and urinary amino-terminal type I collagen telopeptide (NTx) marker levels. We hypothesized that heredity plays a significant role in bone formation and degradation in men. Dietary calcium, smoking, and physical activity were expected to have a lesser influence on these markers.

MATERIALS AND METHODS

Subjects

Male twin pairs (147 MZ and 153 DZ) were selected from the Finnish Twin Cohort, which contains virtually all Finnish twin pairs born before 1958 and living in 1975. The twin pairs were initially recruited to differ in common exposures suspected of influencing back and other musculo-skeletal degenerative changes and back symptoms. Selection criteria were based on discordance in occupational materials handling, sedentary work, exercise participation, vehicular vibration and cigarette smoking. Extensive description regarding the questionnaires has been reported in detail earlier. [19] Zygosity was determined by a validated questionnaire method [20] and has been confirmed by subsequent DNA analyses in these pairs.

A previous analysis regarding representativeness of the selected MZ pairs as compared to all MZ male pairs from the Finnish twin cohort has been performed. [21] Twins originate from all social levels and all regions of Finland. No significant differences were observed compared to the referents for level of education, social class, occupational category, outdoor vs. indoor work leisure-time physical activity, history of work-incapacitating neck, shoulder or back pain, smoking status and life satisfaction. The only statistically significant differences observed between study pairs and the base population of twins in the Finnish Twin Cohort, were work status and physical loading at work, which is probably due to subjects' selection partly on these characteristics. Subjects were more likely to be working and have higher physical work demands. [21] In the current study subjects with medical conditions or taking medications thought to influence biological variation of markers and affect bone turnover (e.g. hypogonadism, tyroid, parathyroid dysfunction, and fracture and prolonged bed rest in the prior year, which is thought to contribute to marker level increases up to 50% [7]) were excluded.

The study protocols were reviewed and approved by the Ethical Committee of the Department of Public Health at the University of Helsinki, the Ethical Committee at the

University of Jyväskylä and the Human Research Ethics Board at the University of Alberta. Participants provided informed consent prior to study participation.

Data collection

An extensive, structured interview was conducted to obtain data on exposure to suspected environmental and behavioral risk factors after study subjects came to a central location in Finland (city of Kuopio). Subjects were queried about lifetime leisure time activities and sport participation frequency, duration (per session) and intensity (light, moderate or strenuous), sitting at work (mean lifetime hours/week), and occupational physical demands (occupational loading scores ranging from 1= sedentary work to 4= heavy physical loading).

Medical history and information on medication use were also obtained through subject interviews. Subjects were excluded from the present analysis if they suffered any kidney or liver disorders (n=4), or if they had a history of the following conditions in the prior year: thyroid or parathyroid disorders (n=1); hormone (cortisone or steroid) therapy (n=23); epilepsy or anti epilepsy medication (n=5); any skeletal disease or fracture (n=31); bed rest of more than 1 month (n=3); or cancer (n=8). Subjects on certain medication in the past known to influence bone metabolism (prescribed for arthritis, elevated blood pressure, or using betablockers, diuretics) were excluded (n=13). After exclusion, 98 MZ pairs and 108 DZ pairs (412 subjects, 68%) were left. 6 MZ and 3 DZ subjects had PINP and ICTP data missing and 73 MZ subjects had NTx data missing. Finally, 403 subjects with data on PINP and ICTP and 339 with data on NTx were available for analysis.

Clinical examinations of each subject were conducted, including dual X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) of the lumbar spine and femoral neck and serum and urine samples (obtained over a one and a half-day period), among other measures. MRI images were obtained using a 1.5 Tesla scanner with a surface coil (Magnetom, Siemens AG Erlangen, Germany). As disc degeneration is less common at L3-L4 levels than in lower levels, mean axial disc area from middle slices of the L2-L4 discs served as an indicator of lumbar spine axial size. Weight was measured using a balance scale, and was recorded to the nearest 0.1 kg, while the standing height was measured in cm. Isokinetic lifting work was assessed by an isokinetic lifting test, a test of fast, maximal lifting at a constant speed from a forward-bent position with knees straight. [22]

Markers of bone synthesis and degradation came from serum and urine specimens stored at -70°C while awaiting analysis. PINP was determined from serum by radioimmunoassay using the propeptide as an antigen (Orion Diagnostica, Finland). The normal concentration in men ranges from 20-76μg/l. [10] Intra- and inter-assay coefficients of variation are 4.6-10.3% and 3.1-10.8%, respectively.

ICTP was analyzed from serum with a radioimmunoassay using polyclonal antibodies against the telopeptide region of type I collagen, which were produced in rabbits (Orion Diagnostica, Finland). The biologic intra-individual CV for ICTP was found to be 10.0%. The analytic intra-assay CV was 5.0-7.9% and 7.0-11.8% for the analytic inter-assay.

NTx was measured in urine using an ELISA resorption assay (Osteomark®; Ostex International) and is normalized to urinary creatinine. The biologic intra-individual CV for NTx was found to be 22.0% with a range of 16-33%. The analytic intra-assay CV was <5%, and the analytic inter-assay <8.0%.

Data analysis

Regression models were fitted using transformed variables. BoxCox transformation was used for PINP and NTx; natural logarithms for the marker values ICTP; and square root for the variables body weight/ axial disc area and isokinetic lifting work in order that the data would approximate a normal distribution. Reported results are age-adjusted on untransformed variables scales. In the regression models, the assumption of independent observations with independent error terms does not hold with twin pairs. Therefore, in order to control for correlations within twin pairs and obtain valid p-values, all statistical tests for means and regression models were performed so as to account for the sampling of twin pairs, and the standard errors and p-values were adjusted appropriately.

The classic twin model is used to estimate the contribution of additive and dominance effects of genes, as well as the contribution of common and unique environment based on similarity of MZ and DZ twins. [23] First step of genetic modeling was to estimate univariate saturated models, which test for the basic assumptions of the twin model, i.e. models of phenotype means, variances, MZ and DZ twin covariances, with age-regression to adjust the variance. Also, assumption of equality of means and variances by zygosity was tested. Twin MZ and DZ twin correlations were calculated by standardizing twin covariances. Monozygotic (MZ) pairs share genetic effects fully (all genes) but also have common environment during childhood and adolescence. In contrast, dizygotic (DZ) pairs share half of their segregating genes in common, but are assumed to have a common environment equal to that of MZ pairs on average. Therefore, a greater similarity of MZ pairs compared to DZ pairs provides evidence for genetic effects in a trait. The extent to which MZ pairs are more than twice similar than DZ pairs permits approximation of additive from dominance effects. [23]

Next, univariate variance component genetic models were fitted to raw data using full-information maximum likelihood estimation. The total phenotypic variance can be decomposed to additive effect of genes taken singly and added over multiple loci (A), dominance effect of genes interacting within loci (D), common environment shared by family members (C) and unique environment not shared by family members (E). One can fit models based on the different combinations of these parameters (e.g., AE, ACE, ADE, and E), but effects due to dominance and shared environmental effects cannot be simultaneously modeled with data limited to that from twins reared together. [23] Thus, it is not possible to distinguish a purely additive genetic effect from the combined influence of additive genetic, genetic effects due to dominance, and shared environmental effects. Because measurement error is included in E, all models always include this component of variance. The most parsimonious model was found based on Akaike's information criterion (AIC). [24] The superiority of alternative, hierarchically nested models was also assessed by Akaike information criterion (AIC; χ^2 – 2 x degrees of freedom). The model with the lowest value of AIC is considered the most parsimonious model (lower AIC indicates better fit).

Bivariate Cholesky decomposition genetic factor model was used to estimate to what degree the genetic (and environmental) effects on one trait are correlated with the genetic effects on another trait and likewise for the environmental effects. Bivariate model-fitting followed the principles used in univariate modelling. After the most parsimonious multivariate model is found, proportions of variance explained by genetic and environmental factors, as well as the correlations between these factors are calculated. The squares of the standardized path coefficients (a, c, d, e) from A, C, D and E, respectively are estimates of the variance components. Bivariate genetic factor models were estimated to quantify the relationship between markers and isokinetic lifting work or body weight/axial disc area (created as a proxy measure of pressure produced by body loading over the individual vertebral body cross-sectional area) in terms of reported genetic effects (A), common environmental effects (C) and unique environmental effects (E) of total variation (i.e. standardized variance component estimates), genetic correlations (r_a) and environmental correlations (r_e) with 95% confidence intervals. The proportion of total genetic (Pa)/environmental (Pe) variation in marker explained by common genetic/environmental variation was also reported.

The STATA statistical software was used for basic statistical analyses [25] and the Mx program, which is software designed for structural equation modelling of twin and family data, was used for genetic analyses. [26]

RESULTS

Meeting the assumption that traits do not differ by zygosity, the means and the variances of the biochemical markers did not differ in MZ and DZ twins, except for the mean of ICTP, which was 3.2 for MZ and 2.8 for DZ twins (Table 1). The MZ twin correlations for ICTP and NTx were about twice the DZ correlations suggesting a genetic influence, while the DZ twin correlation for PINP was higher than half of the MZ twin correlation (Table 2).

In the univariate genetic modeling for PINP marker, ACE was the best fitting model, CE the second best and the third best was the AE model (Table 3). If the parsimony of models is taken into account, AE and CE models gave adequate explanation of underlying variability, but CE was not considered biologically plausible given that genetic effects have been observed in women. Most likely there is a combination of additive genetic and common environmental effects. Additive genetic effects accounted for 29% (95%CI 0-67%) (under the ACE model) to 63% (95%CI 51-72%,) (under the AE model) of the variance in PINP.

For NTx and ICTP, the best fitting (and most simple) model was AE (compared to ACE model), whereas the CE and E models did not fit well at all (Table 3). Additive genetic effects accounted for 65% of the variance in NTx and ICTP (Table 4).

Age, body weight/axial disc area, isokinetic lifting work, sitting at work (mean lifetime hours/week), job heaviness score, heavy leisure time activities, smoking (pack years) calcium intake and alcohol were considered in the regression models for markers based on all individuals. In the models the only statistically significant associations with NTx were age explaining 1.9% ($p<0.05$) and weight/disc area explaining 3.7% ($p<0.01$) of NTx variance. Similarly, age explained 1.7% (<0.05), weight/disc area 3.5% (<0.01) and isokinetic lifting work 1.4% of the variance in PINP levels ($p<0.05$). Calcium was not significantly associated with the markers PINP, ICTP and NTx (r^2= 0.002; 0.0008; 0.002, $p>0.05$), nor was alcohol (r^2= 0.008; 0.002; 0.01, $p>0.05$) or smoking (r^2= 0.002; 0.003; 0.001, $p>0.05$).

In the bivariate models for PINP levels vs. weight/axial disc area, MZ cross-twin cross trait correlations (e.g. correlation of PINP from one twin with weight/axial disc from the other twin) were quite high (-0.23 and -0.36) compared to DZ cross-twin cross trait correlations (-0.06 and 0.03) suggesting genetic effects on common variance. PINP vs. isokinetic lifting work and NTx vs. weight/axial disc area: phenotype correlations and cross-twin cross trait correlations were all quite low suggesting very low or even absent genetic or environmental effects on common variance, which was confirmed in the bivariate genetic factor modeling (results not shown). In the bivariate genetic factor modeling the only significant correlation was the one for PINP vs. weight/axial disc area (-0.37 (-1,-0.05) in ACE model). In the most parsimonious model from the bivariate genetic factor analysis, 13% of the genetic variation in PINP was explained by common genetic variation with weight/axial disc area ($p<0.05$) (Table 5).

DISCUSSION

The findings of this study support a dominant role for heredity in the variation of the bone resorption marker (NTx and ICTP) levels in men, while genetic factors may contribute less for PINP, a marker of bone formation. We are not aware of any prior studies conducted in men that address the influence of genetic factors on bone turnover marker variability.

Genetic factors contributed significantly to bone turnover marker variation in most studies in women. [13-16] The present results in men regarding twin intraclass correlations (rMZ= 0.60 vs. rDZ= 0.34) were similar to a study of untreated postmenopausal women, where MZ twin correlations for NTx, an index of bone resorption, exceeded that of DZ pairs (rMZ= 0. 62 vs. rDZ= 0.34), suggesting that genetic factors are influencing the markers and that bone turnover is heritable[13]. In these twin studies heredity estimates ranged from 29% for serum osteocalcin [16] to 95% for bone formation marker PICP [15]. The heritability estimates in the present study were within the range or slightly lower than in earlier studies of biochemical markers in women. Similarly to one of the studies in women [15] which showed that bone resorption markers are heritable, with genetic factors accounting for 64% in the variance in ICTP, in our study additive genetic effects explained 65% of the variance. Heritability estimates for NTx (a marker of bone resorption) slightly exceeded those found in a study in untreated postmenopausal women (55%). [13] On the other hand, in our study genetic factors contributed less for the marker of bone formation, PINP, which was 29% under the ACE model to 63% under the AE model, compared to over 90% of the variance explained in PICP in studies of women [13,15] suggesting that the genetic effect on collagen I synthesis might be different in men and women. However, these authors have reported heritability estimates based on Falconer's formula: $H^2= 2(rMZ-rDZ)$, [27] which assumes equal phenotypic variances in MZ and DZ twin pairs. However, the variance of a trait for MZ twins is the sum of common and unique environmental effects variance, while for DZ twins is the sum of variances due to genetic, common and unique environmental effects. Therefore, Falconer's formula [27] is only a very crude initial tool, and can lead to biased estimations. For example, if rMZ=0.8 and rDZ=0.2, Falconer's index of heritability would give 2 (0.8-0.2) = 1.2, suggesting that the proportion of variance ascribed to genetic factors is larger than the total variance (120%). The heredity estimates are also conditional on the choice of genetic model and twin analysis program, so the gender difference may not be all that large. Differences in the study populations also may contribute to differences in the heritability estimates. One of the studies in women [15] included postmenopausal twins recruited from the Australian Twin Registry through the local media and had a much smaller sample size, and the other study [13] consisted of a relatively small number of DZ pairs recruited from a national twin registry and media campaign, as compared to the present study sample of male twin pairs selected from the population based Finnish Twin Cohort (Table 6). A strength of the present study is the representativeness of the sample of the population of Finnish men and the availability of extensive interview data on potential covariates. However, heredity estimates in all twin studies hold only with assumptions of the classic twin study design that genetic factors and environmental factors are not correlated, there is no genotype and environment interaction and there is random mating in the population with respect to the traits under study. [23] Another assumption is that both types of twin pairs (MZ and DZ) share fully and equally in magnitude common environmental effects relevant to the trait being studied.

The results of twin studies have prompted the search for candidate genes for different aspects of bone turnover and suggest that there are new candidate genes to be found. Some candidate genes were studied in association with the markers NTx and PINP in men, like estrogen receptor gene, ER (Xbal and Pvull polymorphisms) and androgen receptor gene, and AR (CAG repeat polymorphism). [28] NTx showed a tendency to high values for the xx and pp genotypes of the ER (the values were higher for x and p alleles than for the X and P alleles, respectively), while PINP was not related to any of these genotypes. [28] The AR gene CAG repeat polymorphism was not associated with PINP, NTx [28], PICP or ICTP. [29] Another study in men found a lack of influence of the ER gene Xbal and Pvull polymorphisms on PICP and urinary NTx. [30]Conversely, women carrying the Px haplotype of the ERα gene and s allele of COLI A1 gene were characterized by higher levels of PICP ($p<0.05$), high PTH and low BMD, suggesting possible interaction between ERα and COLI A1 Sp1 polymorphism and resulting in altered production of the α1(I) chain (the protein product) and greater risk of low BMD and higher bone turnover. [31] Because of the complex polygenic etiology of osteoporosis, some of the polymorphisms studied might be only a modest component in bone turnover variation or might be masked by other factors, including environmental influences. Results of possible candidate genes for biochemical markers need to be replicated in further studies.

The sample size of the present study of about 400 was maximized with respect to examining environmental and behavioral effects by using a twin study with comprehensive data on covariates, which increases the power to detect smaller effects. The present study found a significant relationship between markers PINP and NTx and body weight/axial disc area, an indicator of force per vertebral body size. Less body weight loading on the spine was associated with higher bone turnover (more bone formation and more bone resorption) which is documented by prospective studies over 2-12 years to be a factor associated with accelerated bone loss. [4,32,33]

Low weight and lean mass were also found to be correlated with low bone mass in men. [6, 34,35, 36] Age-related bone loss that occurs in both men and women later in life is thought to be related to the decline in muscle mass with advancing age. [37] In an earlier study using a subset of subjects from the same Finnish Twin Cohort, fat-free measures representing higher bone and muscle mass were associated with less bone resorption (as estimated by NTx) and higher BMD values. [6] As fat-free measures were more strongly and independently associated with BMD and NTx than body weight, these relationships were not explicable simply in terms of weight-bearing effects alone, but also possibly by greater physical activity and capacity to generate muscle forces across bone.

The available data in this study allowed the exploration of possible pathways through which genes and environment influence the markers. The genetic correlations indicate the presence of underlying genetic factors common to PINP and body weight/disc axial area. It appears that approximately 13% of the genetic influences on PINP, a marker of bone formation under the ACE model (or 6% under the AE model) are due to the same genes influencing weight/disc axial area. This indicates that individual body weight loading on the spine is not likely to be one of the major pathways through which genes influence the bone formation marker. Environmental factors influencing body loading on the spine axial area did not contribute to the variance in PINP. Although the marker levels seem to be heritable, the genetic loci influencing the marker NTx and loading on the spine by body weight appeared also to be largely independent, as were the environmental factors. This indicates that genetic influences on bone resorption are unlikely to be a result of genetic regulation of individual body weight relative to skeletal size.

In this study we explored behavioral factors like the effect of calcium, smoking and alcohol and found that these factors were not significant determinants of biochemical marker levels in men. These results are concordant with findings of an earlier analysis of a subset of the male MZ twins that participated in the present study, where no statistically significant associations were found between BMD and cigarette smoking, coffee intake and alcohol consumption, while dietary calcium explained only 1% of the variance of BMD at the femoral neck and 0% at the lumbar spine, [38] as well as another study [39] in which childhood milk consumption explained 2% of BMD in the adulthood. Other studies in women [18] found that calcium supplementation was associated with 18% decreased levels of NTx, alcohol intake with 25% lower NTx levels, while current smoking with statistically significantly reduced levels of bone formation (PICP) markers compared to nonsmokers. However, no such pattern was observed in males. [40] Men with regular alcohol consumption as compared to those with moderate alcohol consumption (two times or less per week) showed significantly reduced levels ($p<0.05$) of the biochemical marker of bone formation PICP and no differences for the markers of bone resorption. Women reporting regular alcohol consumption (more than two times per week) showed lower levels of both serum and urinary biochemical markers [40] than their moderate drinking counterparts.

Although some of the studies showed that calcium supplementation and regular alcohol consumption could be beneficial for bone metabolism in women while smoking is detrimental, there is an inconsistency in results and more insights on the influence of these factors on bone metabolism in men are needed. The short interval in collecting the measures of behavioral variables (within weeks), the negative bone balance present in postmenopausal women, and the large intra-individual variability and diurnal variation of markers could be factors that might have contributed to inconsistency in the results. The least significant change (LSC) has been estimated to be in the order of 30-40% for serum markers [41] and it is higher for urine markers, surmounting the biological variation within an individual which can range between less than 10% for serum markers and 30% for urine markers. [42] However, besides the biologic variability, the variability of the markers could also be influenced by the analytical (measurement) variability for the assays.

CONCLUSIONS

The study showed that bone markers are highly heritable in men, with additive genetic effects explaining two thirds of the variance in bone resorption markers NTx and ICTP, and similar or lower variance in the bone formation marker PINP, depending on the model used. The genetic loci influencing the marker PINP or NTx and body weight/disc axial area, although related in part, appeared to be largely independent, indicating that genetic effects on bone formation and resorption are unlikely to be a result of genetic regulation of individual body weight. The genetic variance in bone markers was largely independent from the other anthropometric and behavioural co-variates studied, supporting the hypothesis that heredity plays a significant role in bone formation and degradation in men, while dietary calcium, smoking, and physical activity after childhood have a lesser influence on these markers.

Table 4.1. Means, standard deviations and test of equality of means and variances, adjusted for sampling of twin pairs

	MZ		DZ		Test of equality of means	Test of equality of variances
	Mean (SD)	N	Mean (SD)	N	p-value	p-value
PINP	44 (15)	190	39 (14)	213	0.09	0.89
ICTP	3.2 (0.85)	190	2.8 (0.75)	213	<0.001	0.95
NTx	36 (16)	123	33 (17)	216	0.22	0.79
Age (years)	48 (7.9)	195	49 (6.7)	216	0.36	0.13
Weight/disc area (%)	1.1 (0.19)	160	1.1 (0.16)	207	0.86	<0.05
Isokinetic lifting work (J)	558 (169)	183	499 (181)	207	<0.01	0.38
Sitting at work (mean lifetime hours/week)	2.1 (2.2)	195	1.9 (2.1)	216	0.32	0.87
Job heaviness score (1-4)	2.5 (0.92)	195	2.4 (0.91)	216	0.23	0.32
Heavy leisure time activities (years)	2.7 (8.1)	195	1.9 (4.8)	216	0.56	0.13
Smoking (pack years)	14 (17)	195	15 (17)	216	0.96	0.28

Table 4.2. Intra-class correlations and N of pairs with data for markers in MZ and DZ pairs

	ICC MZ	ICC DZ
PINP	0.620	0.432
N(pairs)	95	106
ICTP	0.620	0.290
N(pairs)	95	106
NTx	0.597	0.337
N(pairs)	61	108

Table 4.3. Comparison of alternative univariate genetic models for biomarkers. Tests of model fit of submodels in comparison to base model with additive genetic effects, common and unique environmental effects (ACE)

	Goodness-of-Fit Tests			
Model	χ^2	df	P-value	AIC
PINP				
ACE	-	-	-	-
AE	3.189	1	0.074	1.189
CE	2.669	1	0.102	0.669
E	69.233	2	<0.001	65.233
NTx				
ACE	-	-	-	
AE	0.000	1	1	-2.000
CE	7.928	1	<0.01	5.928
E	40.363	2	<0.001	36.363
ICTP				
ACE	-	-	-	
AE	0.000	1	1	-2.000
CE	12.693	1	<0.001	10.693
E	61.993	2	<0.001	57.993

Table 4.4. The best fitting, most parsimonious univariate genetic models for biochemical markers

	Components of variance estimates		
Model	Additive Genetic Effects	Common (Shared) Environmental Effects	Unique Environmental Effects
PINP			
ACE	0.29 (0.00 , 0.67)	0.32 (0.00 , 0.59)	0.39 (0.29 , 0.52)
AE	0.63 (0.51 , 0.72)	-	0.37 (0.28 , 0.49)
NTx			
AE	0.65 (0.50 , 0.76)	-	0.35 (0.24 , 0.50
ICTP			
AE	0.65 (0.53 , 0.74)	-	0.35 (0.26 , 0.47)

Table 4.5. Bivariate genetic factor modeling

Phenotypes	r_a	Pa	r_e	Pe
PINP vs. Weight/disc area (ACE for PINP)	-0.37 (-1, -0.05)	0.13	-0.09 (-0.28, 0.09)	0.01
PINP vs. Weight/disc area (AE for PINP)	-0.24 (-0.44, -0.04)	0.06	-0.11 (-0.29, 0.08)	0.01
NTx vs. Weight/disc area	0.17 (-0.09, 0.42)	0.03	0.20 (-0.12, 0.48)	0.04

r_a = Additive genetic correlation
r_e = Unique environmental correlation
Pa= Proportion of total genetic variation in marker variable explained by common genetic variation with the other phenotype in model
Pe= Proportion of unshared environmental variation in marker variable explained by common unshared environmental variation with the other phenotype in model

Table 4.6. Intra-class correlations, heritability estimates, age and N of twin pairs for this and other classic twin studies on the heritability of markers

	ICC MZ	ICC DZ	Heritability estimates (95%CI)	Age (Mean, SD) MZ/DZ	N pairs MZ/DZ
PINP present study	0.62	0.43	0.29 (0.00, 0.67) ACE 0.63 (0.51, 0.72) AE	48 (7.9)/ 49 (6.7)	95/106
PICP (Garnero et al.1997)	0.82	0.33	0.99 (0.51-1.47)^{a}	61 (4.8) / 60.7 (5.6)	61/59
PICP (Tokita et al.1994)	0.78	0.31	0.95^{b}	48.4(13.7)/45.6(10.3)	42/40
ICTP present study	0.62	0.29	0.65 (0.53, 0.74)	48 (7.9) /49 (6.7)	95/106
ICTP (Tokita et al. 1994)	0.68	0.36	0.64^{b}	48.4(13.7) /45.6(10.3)	42/40
NTx present study	0.60	0.34	0.65(0.50 , 0.76)	48 (7.9) /49 (6.7)	61/108
NTx (Garnero et al.1997)	0.62	0.34	0.55 (-0.02-1.11)^{a}	61 (4.8) / 60.7 (5.6)	61/59

$^{a, b}$ The index of heritability was estimated by the Falconer's formula: $H^2 = 2(rMZ-rDZ)$

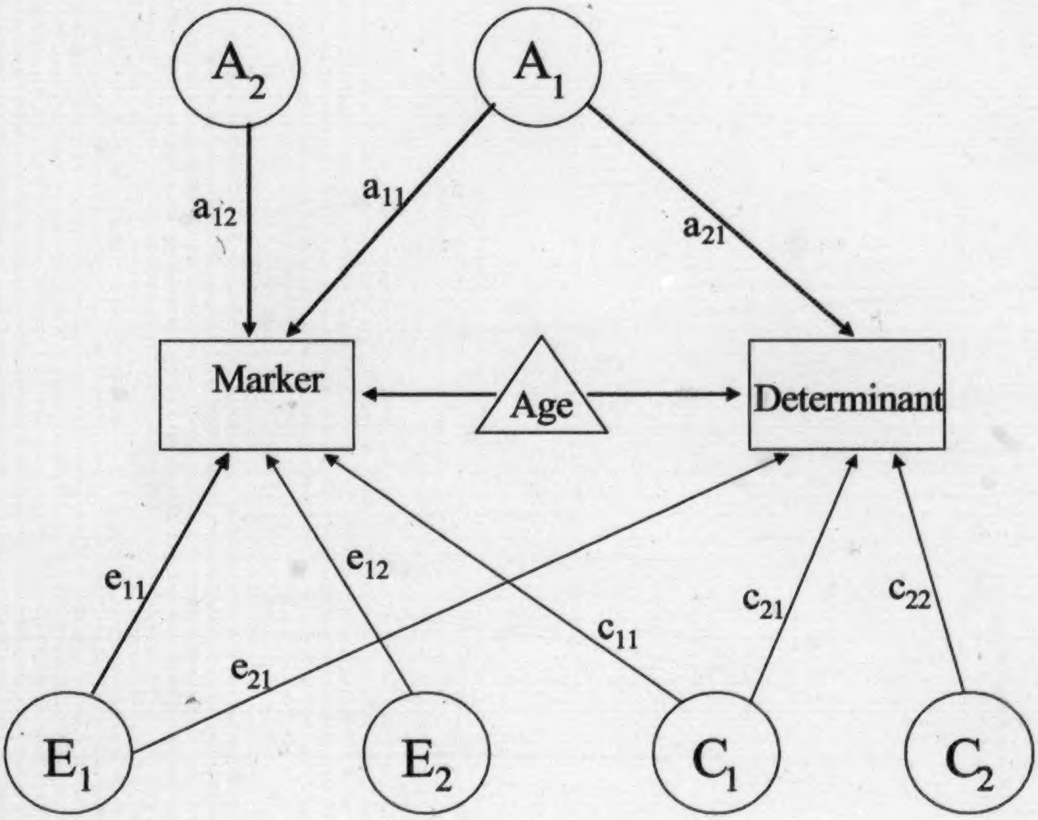
A2
A1
a12
a11
a21
Marker
Age
Determinant
e11
e12
e21
c11
c21
c22
E1
E2
C1
C2

Figure 4.1. Graphical presentation of bivariate genetic model for marker variable and determinant

A= additive genetic effects; C= common environment shared by family members; E= unique environment not shared by family members

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CHAPTER 5: THE INFLUENCE OF DEGENERATIVE MRI FINDINGS ON DXA MEASUREMENTS AND MARKERS OF BONE TURNOVER

INTRODUCTION

The femoral neck and vertebra are the most important sites for clinical assessment in osteoporosis. However, bone mineral density (BMD) measurement obtained from dual-energy X-ray absorptiometry (DXA), the current standard method used in osteoporosis diagnosis, is influenced by bone size, as DXA area measurements cannot correct for antero-posterior depth.[1] Also, the reported discordance between measurements taken at different sites and the difficulties in standardization between DXA instruments can lead to misdiagnosis, particularly if using a single site measurement for BMD. [1,2,3,4]

The integral measurement of cortical and trabecular bone is an important limitation,[1] as different changes might occur in each of the bone components. There are also important measurement challenges at the lumbar spine in the older population receiving DXA, such as contour and shape changes due to localized compression or remodeling, degenerative changes (osteophytic formation and endplate sclerosis), and the presence of aortic calcifications that could falsely elevate lumbar spine BMD measurements in older populations. [1,5] For example, when measuring only trabecular bone with quantitative computed tomography (QCT), BMD was found to decrease with age in subjects with and without large osteophytes, and was significantly higher in men without fracture than in those with fracture. Conversely, integral BMD decreased with age only in subjects without large osteophytes, and no significant difference in BMD between men with and without fracture was found. [6]

As an alternative or adjunct to conventional densitometry, which gives no data on bone quality, and to QCT, which is limited by very high radiation exposure and cost, [7] it has been suggested that bone trabecular structure and quality could also be assessed by MRI T1 and T2 relaxation times as an adjunct measure.[8] T1 seemed not to be influenced by bone size, and was found to be a better predictor for true bone mineral density than T2. [8] Therefore, MRI using T1 relaxation time could be useful in assessing bone morphology and remodeling, including osteophytes, endplate sclerosis and concavity and vertebral height changes. Although there is some data on osteophytes and endplate sclerosis, further data is necessary to explore the nature of these findings and the influence on DXA measures.

Although the mechanisms were not fully elucidated, it has been shown that an increase in fat content accompanies bone loss, and that bone marrow cells are implicated in bone remodeling. [9] In an experimental study on excised human vertebrae, a good correlation between T1 relaxation time and increased content of hematopoietic marrow and abnormally increased bone mineral suggested that MRI can contribute to the assessment of bone quality. T1 was found to be shortened with increasing quantities of fat, and the presence of fat caused a decrease of integral BMD. [9] Also, other studies showed that vertebral marrow fat content is related to bone density. Griffith et al. (2005) [10] found an increased fat content in osteopenic and osteoporotic vertebral marrow measured by hydrogen 1 MR spectroscopy correlated with reduced bone density in men. Another study suggested that bone marrow fat and BMD may be weakly or only partially related. [11]

It has been also suggested that biochemical markers of bone remodeling could provide useful information regarding the status of bone loss, and that biochemical markers and BMD may provide better prediction of future fracture risk than BMD alone.[12,13] Bone formation markers are proteins released from osteoblasts, like the amino- and carboxyl-terminal procollagen I extension peptides (PINP, and PICP, respectively). During bone resorption, amino- (NTx) and carboxyl-terminal telopeptides (ICTP) of collagen are discharged having cross-links attached. It is possible to detect changes in bone turnover within 3 months using biochemical markers rather than 1-2 years as required for BMD measurements. [12] However, many studies found weak [14,15,16,17] or no significant relationship between markers of bone remodeling and BMD. [18]

The objectives of this study were to explore degenerative features at the vertebral body using conventional MRI in relation to DXA measures at the lumbar spine and femoral neck to examine the nature of these findings, and their influences on bone measurement by DXA and bone turnover markers. Among the vertebral findings of interest that were hypothesized to be primarily atrophic in nature are signal intensity variations associated with fatty infiltration and endplate concavity. We expected that age-related substitution of trabecular bone of the vertebral body by fat as indicated by T1 relaxation time, a greater concavity of vertebral endplates, and low vertebral height will be reflected in bone degradation as indicated by low BMD. Osteophyte formation and endplate sclerosis are hypothesized to represent hypertrophic changes and to be a cause of increased bone density measurements. Therefore, they are expected to correlate positively with higher BMD measurements. The correlation of DXA with the markers of bone turnover is expected to increase when adjusting for degenerative findings based on MRI measures.

As MRI, DXA and biochemical markers provide complementary information regarding bone properties, the examination of their relations could be helpful in the diagnostic process and prognosis of metabolic bone diseases. Therefore, the study could lead to further development of adjunct measures for bone strength and bone mass assessment.

MATERIAL AND METHODS

A cross sectional design was used.

Subjects

The study sample was selected from the Finnish Twin Cohort. Subjects originate from all social levels and all regions of Finland. A previous analysis regarding representativeness of the selected subjects as compared to all male pairs from the Finnish Twin Cohort has been performed. No significant differences were observed compared to the referents for level of education, social class, occupational category, outdoor vs. indoor work, leisure-time physical activity, history of work-incapacitating neck, shoulder or back pain, smoking status and life satisfaction. There were no statistically significant differences observed between study subjects and the base population of the Finnish Twin Cohort on any variables examined, with the exception of work status and physical loading at work, probably due to subjects' selection partly on these characteristics. Subjects were more likely to be working and had slighlty higher physical work demands.[19]

The sample for this study was composed of 150 males 35–70 years old at baseline, with data available for all markers and MRI. Subjects were excluded from analysis if they had a history of the following: any chronic kidney or liver disorders (3), or if they had a history of the following conditions or medications in the prior year: any skeletal disease or fracture (21); or bed rest of more than 1 month (3); thyroid or parathyroid disorders (0); hormone (cortisone or steroid) therapy (4); active cancer (3); epilepsy or anti epilepsy medication (3). These factors are known as causes of secondary osteoporosis and consequently they lead to a decreased bone mineral density at different sites of the skeleton. Some of the subjects had more than one condition, therefore a total of 24 subjects were excluded, leaving 126 (84%) subjects with MRI image data. Marker data were available for 119 subjects. Possible unreported incident vertebral fractures were visually assessed by semiquantitative inspection using criteria outlined by Genant et al (1993) [20] as well as examination of associated signal intensity abnormalities on MRI images, to ensure that subjects with recent fractures are not likely to be included in the final sample. 17 subjects (in addition to the fractures identified through subject medical histories) met these criteria and were excluded, leaving 102 subjects with data available that were included in the analyses.

Data collection

Data collection involved a structured interview including medical history, MRI of the spine, DXA at the lumbar spine and hip, and serum and urine samples collected in the morning. The markers from serum and urine and MRI measures were collected under investigational review board approval from the clinical sites. Serum and urine specimens were stored at -20°C at the site hospital. In the present study the laboratories where the assays for the biochemical markers mentioned were developed were used for analysis of samples and intra and inter- assay coefficients of variation are known.

Bone mineral density was measured with dual energy X-ray absorptiometry (DXA; Lunar DPX, Madison, WI), at the L1-L4 vertebrae and right femoral neck and has been described in detail earlier. [21] The coefficient of variation for BMD measurements was 0.9% for the spine and 1.5% for the femoral neck. National, ethnic mean values for BMD in 20-29 year old men are 1.06 g/cm² for femoral neck and 1.23 g/cm² for lumbar spine. Standard deviations for BMD are 0.14 g/cm² for the femoral neck and 0.15 g/cm² for lumbar spine.

Magnetic resonance images of subjects' lumbar spines in the midsagittal plane were obtained using a Siemens 1.5T MRI Scanner with a surface coil. The subjects were imaged after one another, and each spent at least 30 minutes lying supine immediately prior to MRI to control for diurnal and activity effects on the disc. Each subject's films were independently assessed by an experienced spine specialist following a set protocol. The assessor was blinded to subject exposures and twinship.

MR images of the spine were assessed using qualitative and quantitative parameters. Repeatability of some qualitative measures was found to be less than ideal, diluting chances of detecting determinants. [22] Therefore, with the exception of superior and inferior endplate sclerosis measures, qualitative parameters were replaced with more quantitative parameters, using custom-designed spine image analysis software, in an effort to achieve more sensitive and repeatable measurements. However, other authors have suggested that a semiqualitative visual inspection might be as valid and reliable as a quantitative method in the assessment of prevalent or incident fractures. [20] All images were assessed using such a method to ensure exclusion of subjects with recent fractures. Any vertebral body with moderate ("grade 2, approximately 25-40% reduction in any height and a reduction in the area 20-40%") [20] or severe deformity ("grade 3, approximately 40% reduction in any height and area"), [20] and with a concomitant area of increased signal intensity ("hot spot") on the MRI image was considered to represent a high likelihood of recent fracture and therefore the subject was excluded from analysis.

The anterior and posterior contours of the vertebrae were segmented manually on the midsagittal T1-weighted image. Two vertical lines added in the spinal canal anteriorly to the spinal cord demarcated an area containing cerebrospinal fluid (CSF). [22] The vertebra was then segmented from the disc by following horizontally the boundary between the vertebral disc and the endplates. The areas created by the intersection of these segmentation lines formed the vertebral and CSF regions of interest from which the measures were derived. The segmented areas on the T1-weighted image were then adjusted by using T2- images, taking advantage of different contrasts (Figure 5.1). Quantitative measures such as anterior and posterior osteophyte areas, endplate concavity area, vertebral medial height, and vertebral body signal intensity were obtained. The specific findings were assessed using mean signal intensity and were adjusted for brightness by using CSF as a reference (e.g. the adjusted vertebral signal intensity was calculated as the mean signal intensity for the whole vertebra divided by the mean signal intensity of the CSF sample at the corresponding level). The concavity of vertebral endplates were measured by area of concavity obtained by connecting the anterior and posterior points of connection between vertebra and the disc through a horizontal line (the area was measured in pixels, 1 pixel^2= 1.02 X 1.02 = 1.04 mm^2). Mean vertebral height was obtained by dividing the sum of vertebral heights at each lumbar level (from a midsagital plane) by 5.

In order to obtain average area for osteophytes at one vertebral level, the areas of osteophytes at the four different corners (2 anterior, 2 posterior) were added, and the sum was divided by the total vertebral area. The data for the lumbar vertebrae were summed and divided by 5 (the number of vertebral levels) to obtain an average osteophyte area for the whole lumbar spine. In order to develop quantitative measures of vertebral contour abnormalities on sagittal MR images, such as the presence of osteophytes near the vertebral corners, Harada et al. (1998) [23] suggested a standardized procedure to identify a position from which to measure the area of contour abnormalities on sagittal images. This procedure is using the mid-point of the posterior vertebral wall of the vertebra above and below a disc, and does not consider that identifying this position is difficult because blood vessels are entering the vertebral body at its midpoint. Therefore, Harada et al.'s method has been adapted by using a point on the anterior and posterior vertebral wall at a position less likely to be influenced by progressive degeneration of the vertebra and the vertebral artery. [24] See Figure 5.2.

The evaluator (O.S.D) had prior experience segmenting over 200 MR images of the spine. The intra-rater reliabilities of the quantitative measures were drawn from a sample of 31 subjects. For the inter-rater reliability measures, two evaluators (O.S.D. and supervisor T.V.) blinded to each other's measurements, as well as to the participants' medical histories, personal profile and risk factor exposures. The intra and inter-reliability of all MRI measures for this study were found to be high (ICC $\geq$ 0.9). See Appendix C.

Biochemical markers. PINP was determined from serum by radioimmunoassay using the propeptide as an antigen (Orion Diagnostica, Finland). The reference interval in men ranges from 20-76 µg/l. [25] Intra- and inter-assay coefficients of variation are 4.6-10.3% and 3.1-10.8%, respectively. PICP serum concentrations were analyzed with radioimmunoassay kits (Orion Diagnostica, Finland). The intra and inter-assay CV were < 6%.

ICTP was analyzed from serum with a radioimmunoassay using polyclonal antibodies against the telopeptide region of type I collagen, which were produced in rabbits (Orion Diagnostica, Finland). The reference interval in men ranges from 1.6- 4.6 µg/l. Intra- and inter-assay coefficients of variation are 2.8-6.2% and 4.1-7.9%, respectively.

NTx was measured in urine using an ELISA resorption assay (Osteomark®; Ostex International) and is normalized to urinary creatinine/24 hours. The biologic intra-individual CV for NTx was found to be 22 % with a range of 16-33%. The analytic intra-assay CV was < 5%, and the analytic inter-assay < 8.0%. NTx values are corrected for creatinine to adjust for the wide-ranging dilution of normal urine spot collections.

Data Analysis

The STATA statistical package [26] was used for data analyses. The α level was set at 0.05 for determining statistical significance. Multiple linear regression analyses were conducted to examine correlations between DXA, age, MRI, and markers. Because of the decreased level of variability between the twins from the same pair, the sampling of twin pairs was accounted for in the analyses. Participants' characteristics, including age, MRI findings, spine and femoral neck BMD, and marker values are summarized in Table 1.

All participants received written information about the study procedures before participation and the study protocols were reviewed and approved by the Ethical Committee of the Department of Public Health at the University of Helsinki, and the Human Research Ethics Board at the University of Alberta.

RESULTS

MRI measures in relation to BMD

Among the quantitative MRI measures, osteophyte size correlated with BMD at the lumbar spine (r= 0.22, p=0.02), vertebrae signal intensity correlated with BMD at the spine (r= -0.33, p<0.001) and femoral neck (r= -0.27, p=0.03). The qualitative measure of endplate sclerosis correlated statistically significant only with femoral neck BMD ($r = 0.20$, $p < 0.05$) and not spine BMD ($r = 0.04$, $p = 0.6$).

The relation of biochemical and age markers to BMD when controlling for MRI measures

In the linear regression analysis, when introduced alone in the model, the markers did not correlate statistically significantly with BMD at the lumbar spine or femoral neck, with the exception of NTx and BMD at the femoral neck ($r = -0.3$, $p < 0.01$). The initial correlation of NTx and vertebral BMD ($r = -0.17$, $p = 0.059$) increased and became statistically significant when any of the following MRI variables were added in the model: osteophytes, endplate sclerosis, vertebral height, vertebral signal intensity, and the measure of endplate concavity ($r = -0.22$ to -0.27, $p < 0.05$) (Table 2). When osteophytes were added in the regression model, the correlation of PICP and BMD at the spine ($r = 0.03$, $p = 0.7$) became stronger ($r = 0.22$, $p < 0.05$). The correlation of BMD at the femoral neck with both PINP ($r = 0.14$, $p = 0.08$) and PICP ($r = 0$, $p = 0.9$), changed in a similar manner ($r = 0.22$, $p = 0.06$ for both). MRI degenerative findings did not correlate with the biochemical markers.

In the linear regression analysis, the initial correlation of age with femoral neck BMD ($r = -0.26$, $p = 0.03$) increased when one of the following variables were added in the model: osteophytes, endplate sclerosis, vertebral height ($r = -0.32$, $p < 0.05$), and vertebral signal intensity ($r = -0.45$, $p = 0.0001$). Similarly, the initial correlation of spine BMD with age ($r = -0.17$, $p = 0.046$) increased when one of the following variables were added in the model: osteophytes, endplate sclerosis, vertebral height, endplate concavity ($r = -0.28$ to -0.32, $p < 0.05$), and vertebral signal intensity ($r = -0.44$, $p = 0.0001$). Initially, in the multiple regression analysis age explained 3% of the variance in spine BMD ($p < 0.05$). When added in the model, vertebral signal intensity added 9%, osteophyte area 7%, and endplate concavity 1% to the explained variance. These variables explained collectively 20% of the variance in spine BMD.

DISCUSSION

The findings of this study showed that degenerative features at the vertebrae such as osteophytes, endplate sclerosis and fatty degeneration affected the overall assessment of bone mineral density by DXA at the spine and femoral neck, diluting the associations between bone mineral density measurements (DXA) and age or biochemical markers. Confirming our hypotheses, the correlation of DXA with age or the markers of bone turnover increased when adjusting for degenerative findings based on MRI measures. For example, the initial correlation of age with BMD at the spine (r= -0.17) doubled when adjusting for either osteophytes or endplate sclerosis (r= -0.3) and substantially increased compared to the initial correlation when adjusting for fatty infiltration (r= -0.44). Age, vertebral signal intensity, osteophytes, and endplate concavity collectively explained 20% of the variance in spine BMD. Possible explanations for these findings are the correlation of age-related substitution of bone trabecular structure by fat at the vertebral body indicated by MRI T1 relaxation time with low BMD, and the correlation of hypertrophic degenerative findings such as osteophyte formation with increased BMD.

It has been suggested that the age-related substitution of red marrow and bone trabecular structure by fat at the vertebral body level may be the cause for diminished T1 relaxation time. [8] This concept is in concordance with the present study results which showed that signal intensity correlated with BMD both at the spine (r= -0.33) and femoral neck (r= -0.27). Previous studies showed that osteoporosis is associated with increased bone marrow fat [27,28] and that increased bone marrow fat may be a risk factor for fractures. [11] It was observed that age related bone loss is accompanied by a progressive increase in marrow fat [27] which might contribute to bone weakening. [11] Possible mechanisms include a direct influence of marrow fat on trabecular bone, [29] increased adipogenesis-osteogenesis competition that might lead to a reduction in the osteoblast population followed by bone loss, [30] and a weak biomechanical support medium by increased bone marrow fat as compared to red bone marrow, which might influence bone strength. [31]

We also hypothesized that vertebral concavity and vertebral height might reflect possible signs of bone loss, but such relationships were not supported by the data. Although previous studies suggested that an accurate measure of vertebral body height could be used to differentiate normal from pathological vertebrae following osteoporotic fractures, [32] the present study was conducted on a population-based cohort of healthy male subjects, without prior fractures. Thus, the lack of association may not be unexpected. In concordance with the present study, where a measure of concavity area did not correlate with BMD, Falazzari et al. (2001) [33] found that vertebral concavity did not correlate with cancelous bone architecture measured through histomorphometric analysis.

Adaptive bone remodeling due to a non-uniform load distribution across the vertebral enplates might contribute to vertebral body and endplate alteration and osteophyte growth. [33] The initial low correlations of DXA with age might be due to bone remodeling such as osteophyte formation, calcifications, or sclerotic changes in the vertebrae which happen in later life, as hypertrophic findings could mask decreased BMD in the trabecular bone. Degenerative findings were found to affect the relation of NTx, a marker of bone resorption, and DXA measures at the lumbar spine and femoral neck. Osteophytes were found to affect the relationship of bone formation marker PICP and DXA measures at the lumbar spine, and of PICP and PINP markers and DXA measures at the femoral neck.

The low correlations found in older subjects from a previous study using the same population-based cohort [17] could be explained by the fact that type I collagen is not bone specific, as it is found in skin, fibro cartilage, tendons, vessels and dentin, [34] the daily variations in individual levels of markers affecting measurement values, and the measurement of current bone activity within the whole skeleton, rather than cumulative outcome at a specific site (as viewed by DXA). Also, low correlations might be due to bone remodeling such as osteophyte formation, calcifications, or sclerotic changes in the vertebrae which happen in later life, as hypertrophic findings could mask decreased BMD in the trabecular bone. The lack of an association between NTx and BMD at the spine found in men over 50 years old (Donescu et al., 2005) was consistent with these possible explanations and results of other studies. [35,36]

The study has several limitations. Although magnetic resonance imaging (MRI) could theoretically overcome some of the weaknesses of conventional radiography (such as lack of reproducibility, difficult visualization of vertebral body outlines and ionizing radiation[37]), and could potentially be an adjunct method to DXA in osteoporosis diagnosis, due to different principles, three-dimensional character, measurement in different planes, many contrast mechanisms, and lack of ionizing radiation, [38,39] conventional MRI techniques used in this study imply T1 and T2 weighted images. Therefore, T1 and T2 components of relaxation are fixed, and consequently no investigation of MRI parameter variation related to BMD or biochemical markers can be performed. Another limitation is the impossibility of assessing trabecular structure, which is currently achievable only with micro MRI, or μCT. Limitations related to biochemical markers include: variations due to diurnal, seasonal changes and sample handling. In order to eliminate their effect, the samples were taken at the same time (in the morning) for all subjects and were analysed using the same laboratory assays.

Taking into account the similarities between the sample and the general population of Caucasian men in the same age range and the sample size, the generalizability of the study to a broader population that meets the inclusion/exclusion criteria is expected. Also, this population is advantageous because of the restricted number of confounding factors as compared to women, where the estrogen influence on bone metabolism can be an important source of bias. However, some studies showed age-related differences in vertebral marrow fat content between men and women [29,40] such that, the results of the study might not be generalizable to women.

As the sample size for this study was relative small (N=110), a cross- or double cross-validation using two large samples, or two samples obtained via random selection procedures from one large sample might be necessary to be conducted in order to achieve confidence in the generalizability of the equation containing specific predictors, [41] such as these degenerative MRI vertebral findings. If these predictors appear to be generalizable, an adjusted measure of DXA for these factors (a predicted BMD) might be a more accurate score than an unpredicted BMD score.

CONCLUSIONS

The present study results point out the influence of degenerative findings based on MRI measurements at the spine such as osteophytes, endplate sclerosis and fatty infiltration on the areal BMD currently assessed by DXA, suggesting that an overall bone assessment such as DXA might not offer an accurate measure of BMD.

Table 5.1. Summary characteristics for participants

VARIABLE	N	MEAN (SD)
AGE (YEARS)	102	49 (8.2)
HEIGHT (cm)	101	175 (6.8)
WEIGHT (kg)	102	80 (12.2)
PINP (µg/l)	101	46.6 (15.5)
PICP (µg/l)	101	138.3 (37)
ICTP (µg/l)	102	2.9(0.7)
NTx (nM BCE/ mM creatinine)	102	38.4(14.6)
SPINE BMD (g/cm^2)	101	1.1 (0.1)
FEMUR NECK BMD (g/cm^2)	101	0.9 (0.1)
OSTEOPHYTES (mm^2)*	102	0.08 (0.02)
ENDPLATE SCLEROSIS (score 0-3)	102	0.4 (0.4)
MEAN VERTEBRAL HEIGHT (cm)	106	11.5 (0.7)
CONCAVITY AREA (mm^2)*	102	6.7 (2.4)
SIGNAL INTENSITY OF VERTEBRA ADJUSTED FOR CSF	101	1.9 (0.3)

*** The digital qualitative measures of area were measured in pixels (1 pixel=1.02 mm^2)**

Table 5.2. Correlations of NTx levels, a biochemical marker, to BMD when adjusting for MRI measures of degenerative changes

	NTx	Age
BMD spine alone (DXA)	**r= - 0.17, p=0.06**	**r= -0.17, p=0.05**
Adjusting for OSTEOPHYTES	r= - 0.27, p=0.04	r= - 0.30, p=0.003
Adjusting for ENDPLATE SCLEROSIS	r= - 0.22, p=0.04	r= - 0.32, p=0.001
Adjusting for VERTEBRAL HEIGHT	r= - 0.24, p=0.05	r= - 0.29, p=0.02
Adjusting for FATTY INFILTRATION	r= - 0.27, p=0.04	r=-0.44, p=0.0001
Adjusting for CONCAVITY	r= - 0.27, p=0.04	r= - 0.28, p=0.02

Figure 5.1 Outlining regions for measures at the lumbar vertebrae

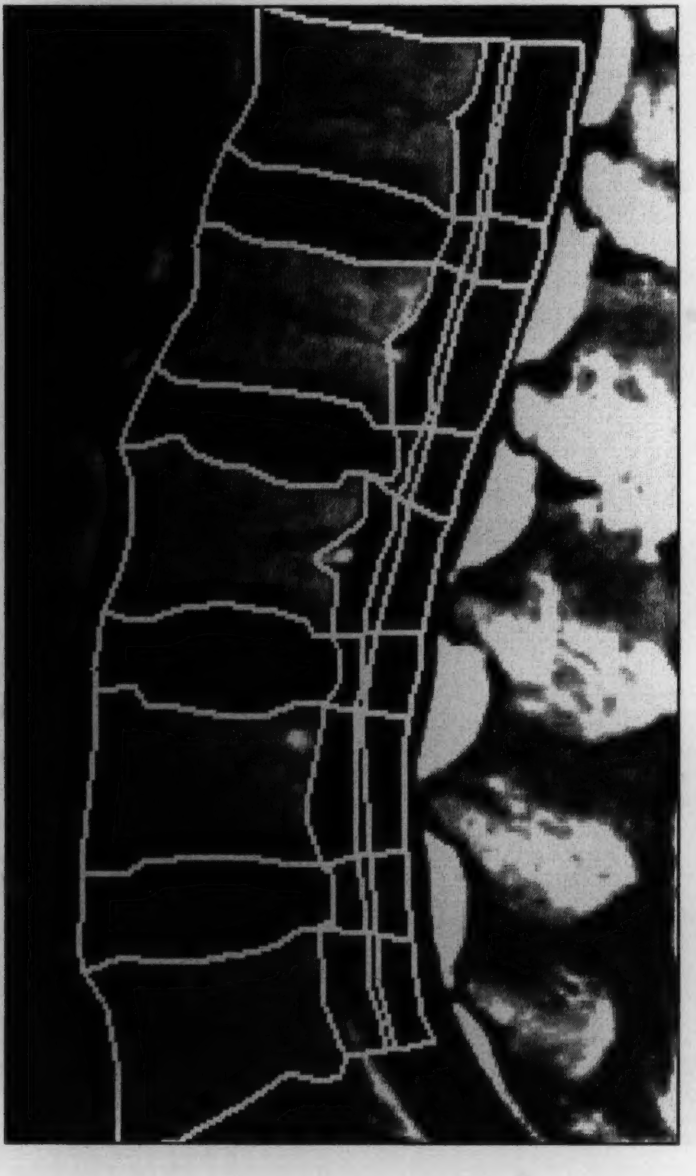
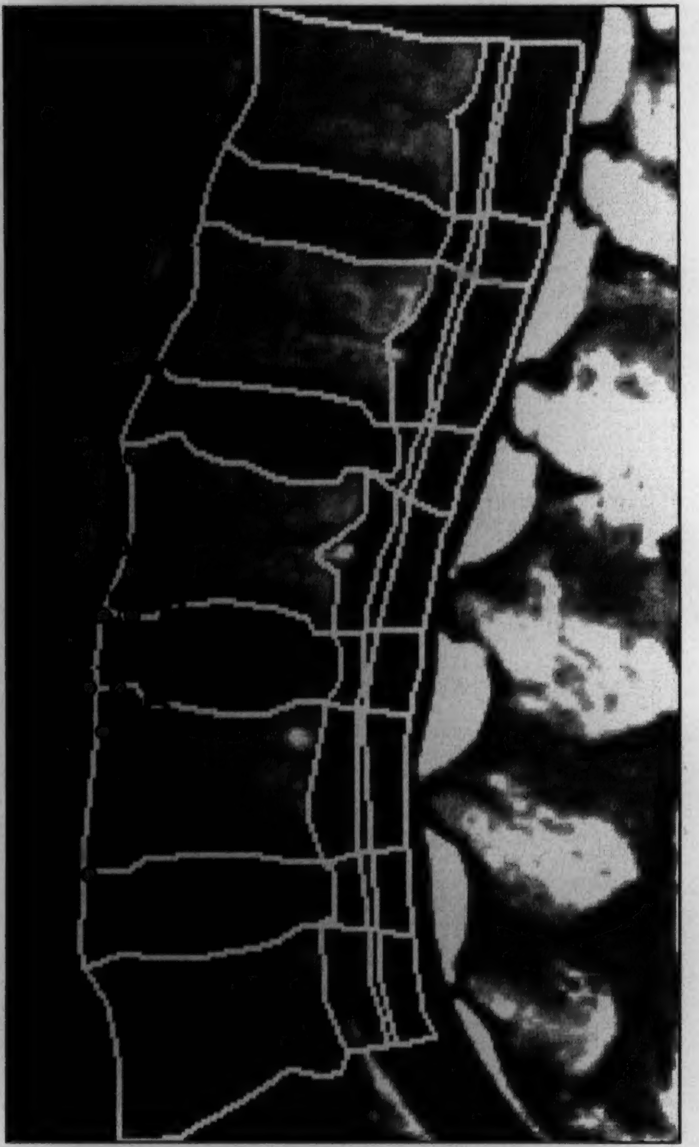


Figure 5.2 Graphical description for osteophytes assessment



Legend. The low anterior osteophyte of a vertebrae was obtained by connecting 3 points: **A** = located on the anterior contour of vertebra, at 20% of the distance connecting the lower (C) and upper anterior corners of vertebra (D); **B** = located at the intersection of the upper part of the disc below, with a "vertical" line connecting the point situated at 20% of the distance connecting the lower and upper anterior corners of that vertebra (A) and a point situated at 20% of the distance connecting the upper and lower anterior corners of the vertebra below (A'); **C** = the lower anterior corner of vertebra, at the intersection of a "vertical" line following anterior contour of vertebra and disc, and a "horizontal" line following the upper part of disc below. The posterior contour of vertebrae is following the vertebral artery in order to exclude it from the measurements of vertebral bone.

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CHAPTER 6: CONCLUSIONS

SUMMARY OF THE MAIN FINDINGS

The first paper (Chapter 2) explored for the first time the relation between anthropometric components and biochemical markers. Of the anthropometric factors studied, fat-free BMI was a stronger determinant for NTx and PINP biochemical marker levels and spine BMD than total weight, while body fat did not correlate with the BMD measures. These findings supported our hypothesis that fat-free parameters reflecting bone and muscle mass play a more important role in bone metabolism than body fat in men.

The classic twin study (Chapter 4) showed that bone markers are highly heritable in men, with additive genetic effects explaining two thirds of the variance in bone resorption markers NTx and ICTP, and similar or lower variance in the bone formation marker PINP, depending on the model used. The genetic variance in bone markers was largely independent from the other anthropometric and behavioral co-variates studied, supporting the hypothesis that heredity plays a significant role in bone formation and degradation in men, while dietary calcium, smoking, and physical activity after childhood have a lesser influence on these markers in men.

The results presented in the first paper (Chapter 2) also showed that urinary NTx was a better indicator of current BMD status than serum PINP, and that NTx levels were significantly different between men with normal and osteoporotic BMD at the femoral neck (cross-sectional study). When the correlations of markers with previous or future BMD changes (over a 5-year period) were further explored (Chapter 3), the results showed that they were not higher than those found in the cross-sectional analysis in men 35 to 69 years old. Baseline PINP, PICP and ICTP marker levels did not predict changes in spine and femoral neck BMD in this group, while NTx levels explained a statistically significant yet quite limited portion of the variance in change in femoral BMD over the prior five years. Thus, we conclude that the usefulness of these markers in predicting age-related change in BMD is of limited value in adult men. Findings from The last study (Chapter 5) suggest that degenerative findings based on MRI measurements at the spine have an influence on the overall bone assessment using DXA, and on the relation of biochemical markers originating from type I collagen with DXA measures at the spine and femoral neck. This might be, in part, one of the reasons for the weak correlation of biochemical markers with BMD.

STUDY LIMITATIONS

Limitations related to biochemical markers

The large biologic and analytical (measurement) variability are some of the markers' limitations. Factors such as age, gender, day to day variation, immobilization, diet, alcohol, smoking, thyroid and parathyroid dysfunction, hypogonadism, different types of medications (e.g. steroids) might affect bone turnover and influence some of the markers biological variation.[1] Most of these conditions were considered in the exclusion criteria (except for alcohol and smoking).

Biological variation is much larger for samples from urine (usually markers for bone resorption) than for serum samples. Markers measured from urine have been reported to vary up to 30%, while markers from serum vary by less than 10% within an individual. [1,2] The day to day variability is also very important because it cannot be controlled, and it causes considerable variation especially to the markers measured from urine. There are also diurnal influences and during the night bone resorption marker levels can reach twice the daytime level. [1] In order to minimize these effects, the samples were taken at the same time (in the morning, after overnight fasting) for all the subjects and were analyzed using the same laboratory assays. Also, the urine samples were normalized for creatinine. However, cross-linked collagen telopeptide markers obtained from urine, such as NTx, show no diurnal variation during daytime and do not require fasting. [3]

Modest relations between BMD and markers may be expected considering that type I collagen is not bone specific and is found throughout the body. Bone collagen assay specificity is influenced by the size of cross-links, the possible existence of variants of type I collagen, various cross-links in soft tissue collagen, structural variability of the cross-linked telopeptides and size of the degradation fragments. [4]

Limitations related to DXA measurements

A limitation of longitudinal studies using DXA measurements is that the measurement error in follow-up studies can be expected to be greater relative to the magnitude of the BMD measure of change than for a single measure of BMD. In order to minimize the differences between baseline and follow-up DXA measurements due to methodological variations, the same type of DXA equipment (GE Lunar corp. Madison, WI) and software for pencil beam densitometers (DPX) were used for baseline and follow-up measurements in this study. However, a possible explanation for the lack of clear association between the markers and BMD change could be that the change in BMD over 5 years was of insufficient magnitude to overcome the dilution of correlations by measurement error in the markers and BMD measurements.

Limitations related to MRI

Measures of trabecular structure, thickness, spacing, and number can be obtained by using high resolution MRI. Unfortunately, these techniques were not available in the present study, as only conventional MRI was used at the time of data collection aimed at investigating disc degeneration, which leads to limitations in studying bone structure. Conventional MRI techniques used in this study imply T1 and T2 weighted images. Therefore, T1 and T2 components of relaxation are fixed, and consequently no investigation of MRI parameter variation related to BMD can be performed. Another limitation is the impossibility of assessing trabecular structure, which is achievable only with micro MRI, or μCT. MRI disadvantages also include contraindications for patients, high cost, reduced availability, longer measurement times, and lower spatial resolution than DXA and computed tomography. [5,6]

Limitations related to study sample

The difference in the results from most studies conducted in men, which found weak or no significant relationship between markers of bone remodeling and BMD, and those in women which found higher correlations, appear to reflect an influence of gender and hormonal differences. The results of several studies suggest that there are factors related to menopause that could contribute to a greater variation in bone metabolism in postmenopausal women when compared to premenopausal women or men. [7, 8] The biochemical markers express bone turnover in the whole skeleton, of which size is considerably influenced by gender. [9, 10] Also, bone metabolism in men is less affected by the influence of estrogen than in women. Therefore, changes in bone mass in women might be of greater magnitude than those found in men, which might not be detectable through statistical methods as they might also be comparable in size to the measurement error.

The role of elevated parathyroid hormone or low testosterone levels in older men should also be addressed by future studies in order to better understand their effect on BMD and biochemical markers of bone turnover, and the variations between age groups. Unfortunately, we did not have data on estrogen, testosterone and other hormones with possible influences on metabolism and muscle strength, and it would be important to see the relation of these hormones with BMD and the biochemical markers in both males and females to further enhance our understanding of mechanisms implicated in bone turnover. Also, the present research is limited by the absence of measures and lack of control of factors, such as insulin-like growth factor-1 or parathyroid hormone, which have been found to correlate with a decline in BMD[11] and the sex-hormone binding globulin, which was associated with hip and spine BMD and CTx marker levels. [12]

The first study (Chapter 2) results showed that NTx levels were different between men with normal and osteoporotic BMD levels at the femoral neck, but not at the spine. The ability of NTx levels to differentiate between BMD categories in this population-based, healthy cohort of adult men could also be limited by the small number of osteoporotic men (only 3 at the spine and 4 at the femoral neck from a total of 145 subjects). Also, the sample size for the study described in Chapter 5 was relatively small (N=110) for 5 predictors (it was recommended to have between 15 [13] and 40 [14] subjects per predictor). Therefore, a study limitation might be the phenomenon of shrinkage (which is described as the difference between the initial R-squared and R-squared of a subsequent sample) that might influence the generalizability of the equation containing the specific predictors.

STUDY STRENGTHS

A strength of the study sample is the representativeness of the sample of the population of Finnish men from which it was drawn and the availability of extensive interview data on constitutional, behavioral and environmental factors (Appendix A). A previous analysis regarding representativeness of the selected MZ pairs as compared to all MZ male pairs from the Finnish twin cohort showed that twins originate from all social levels and all regions of Finland, and there are no significant differences compared to the referents for level of education, social class, occupational category, outdoor vs. indoor work leisure-time physical activity, history of work-incapacitating neck, shoulder or back pain, smoking status and life satisfaction. The only statistically significant differences observed between study pairs and the base population of twins in the Finnish Twin Cohort, were work status and physical loading at work, which is probably due to subjects' selection partly on these characteristics. [15] Taking into account the similarities between the sample and the general population of Caucasian men in the same age range and the sample size, the generalizability of the study to a broader population that meets the inclusion/exclusion criteria is expected. Also, this population is advantageous because of the restricted number of confounding factors as compared to women, where the estrogen influence on bone metabolism can be an important source of bias.

The power to detect smaller effects was particularly increased when using the twin study design. Twins are uniquely matched for age, sex and multiple confounding variables that can be controlled for. Therefore, the twin studies enable a powerful design using a relatively small sample. Also, the difficulty to reliably measure behavioral factors over long periods that lead to inconsistent results in studies of lifestyle factors and bone loss, [16] might be overcome by using comprehensive interview data on different co-variates.

Biochemical marker levels can change between 30-70% during lifestyle changes,[17] and over shorter intervals (within weeks) as compared to BMD measurements. Therefore, their assessment can offer an alternative to measuring changes in bone mass.

Biochemical markers are connected to aspects of bone quality rather than to bone mass. Biochemical markers of bone resorption were found to be useful predictors of fracture risk, independent of BMD, as seen in population studies of elderly women. [18,19]

The markers might capture more dynamic bone properties like current bone activity within the whole skeleton or architectural characteristics independent of BMD measurements. Therefore, they could be a contributing factor in risk of fracture even if bone mass measurements of a specific site viewed by DXA are unchanged or do not predict change in BMD.

Magnetic resonance imaging (MRI) could theoretically overcome some of the weaknesses of conventional radiography (such as lack of reproducibility, difficult visualization of vertebral body outlines and ionizing radiation, [20] and could be a potentially adjunct method to DXA and CT in osteoporosis diagnosis, due to different principles, lack of ionizing radiation, three-dimensional character, capabilities of taking measurements in different planes, and many contrast mechanisms. [21,22]

FURTHER DEVELOPMENT

As noted at the Canadian Consensus Conference on Osteoporosis (2006), [23] bone turnover marker cut-offs related to fracture risk are yet to be established. However, in the "Guidelines for the use of biochemical markers of bone turnover in osteoporosis" (2004), [24] reference ranges and some cut-off levels for markers were provided as indicative of bone disease or high fracture risk. In order to reduce the influence of circadian variability on clinical interpretation, the sampling needs to be rigorously controlled: early morning (for serum collection: 9AM; for urine collection: first or second voided urine, followed by creatinine correction) after an overnight fast. [25] However, the overnight fast is not required for NTx (from serum or urine) and PINP (serum) as as they are not affected by diet, but repeated measurements should be taken under the same test conditions. [24] Both bone formation and resorption markers should be measured prior to therapeutic interventions, as the degree of bone resorption might outweight that of bone formations. [24]

The most recent Canadian Consensus Conference on Osteoporosis report (2006)[23] suggested that biochemical markers "can be used to rapidly assess adherence and effectiveness of pharmacological interventions", but cannot be recommended for prediction of bone loss until more data on clinical applications is available.

The last study results in this thesis suggested that areal BMD currently viewed by DXA does not offer an accurate measure of BMD, as various factors (e.g. osteophytes, endplate sclerosis, fatty infiltration) were found to influence the overall measure of bone using DXA. As the sample size might be a limitation, a cross- or double cross-validation using two large samples, or two samples obtained via random selection procedures from a large sample would be necessary to ensure the generalizability of the equation containing specific predictors. If these predictors and the magnitude of their effects appear to be generalizable, an adjusted measure of DXA for these factors (a predicted BMD) might be a more accurate or meaningful score than an unadjusted BMD score.

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APPENDIX A: INTERVIEW SURVEY FOR TWIN SPINE STUDY

Subject No. ______

Today's date: ____/____/____ (day/mo/yr)
Examiner _________
Coder ____________

TWIN SPINE STUDY (INTERVIEW SURVEY)

1. Name: ____(first, last)

2. Date of birth: ____/____/____ (day/mo/yr) Age ______yrs.
BP___/___

3. Current marital status:

1	never married	4	living with someone (unmarried)
2	married	5	divorced or separated
3	re-married	6	widow/widower

4.1 Education Level:

1 partial elementary
2 complete elementary
3 incomplete middle school
4 complete middle school
5 incomplete high school
6 complete high school

4.2 Professional Education

1 none
2 professional course
3 apprenticeship
4 trade school
5 trade school plus
6 trade institute
7 University
8 other: _________________________

LIFE SPAN

1	elementary school	7	marriage or engagement
2	middle school	8	divorce
3	trade school	9	move
4	high school graduation	10	birth of child
5	post-high school studies	11	began profession
6	military service	12	pension date

HEALTH HISTORY ***(Health related questions from the standardized interview):***

General Health

5. How do you evaluate your health today compared to others of your age?
    1 much better
    2 somewhat better
    3 same
    4 slightly worse
    5 much worse

6. How is your health compared to your twin brother's (currently)?
    1 much better
    2 somewhat better
    3 same
    4 slightly worse
    5 much worse

Do you have or have you had any of the following diseases?

(check all that apply, if any responses are positive, then explain when how, where the condition was recognized, what the treatment was, when it started, how long it lasted)

8. chronic bronchitis, emphysema, or chronic cough (with sputum) diagnosed by a physician
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

9. asthma diagnosed by a physician
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

10. other diagnosed respiratory diseases
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

11. myocardial infarction
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

12. angina pectoris
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

13. cardiac insufficiency (ie. valve problems)
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

14. cardiac arhythmia
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

15. other heart diseases
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

16. blood pressure
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

17. arterial thrombosis or neurogenic intermittent claudication
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

18.1 stroke or other ischemic brain disorders
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

18.2 migraine headaches
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

19. During the past year, about how frequently have you had headaches?
1 daily
2 not daily, but at least once a week
3 not weekly, but at least once a month
4 several times a year
5 2-3times/year
6 once a year
7 none at all

20. Have you been diagnosed as having epilepsy
1 no 2 yes, If yes, when was it discovered ______

Was it treated? 1 no 2 yes

21. other neurologic diseases
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

22. eye disease
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

23. ear disease (including hearing impairment)
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

24. mental problems
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

25. severe psychiatric disease
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

26. diabetes
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

27. thyroid gland disorder
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

28. parathyroid gland disorder
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

29. endocrine (hormonal secretion) disorders
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

30. stomach disorders
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

31. intestinal disease
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

6

32. liver disease
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

33. During the past year, about how frequently have you had stomach upset?
1 daily
2 not daily, but at least once a week
3 not weekly, but at least once a month
4 several times a year
5 2-3times/year
6 once a year
7 none at all

34. If you have had stomach upset, explain
____
____
____
____

35. Has a doctor discovered you have lactose intolerance?
1 no 2 yes If yes, when ___________

36. Has the use of milk products caused you stomach upset?
1 no 2 yes

37. Have you restricted the use of milk products (milk, ice cream, yogurt, whole milk or sour milk)
because of stomach upset?
1 no 2 yes, If yes, when______________________

38. Have you been diagnosed as having kidney disease?
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

39. Have you been diagnosed as having arthritis?
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

40. Have you been diagnosed as having any skeletal diseases?
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

41 Have you had fractures?
1 no 2 yes, If yes, what did you fracture and when?

(note left or right)

Location	Date
1. spine	________
2. arm	________
3. forearm or wrist	________
4. hand	________
5. pelvis	________
6. hip/thigh	________
7. leg or ankle	________
8. foot	________
9. other, what?	
Total______	

42. Have you been diagnosed as having anemia?
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

43. cancer?
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

44. other disease? :____________________________
1 no 2 yes, If yes, when was it discovered ______
Was it treated? 1 no 2 yes

45. Explain the course of the diseases (when was the onset, who diagnosed it, what was its course)

____

____

46. For the diseases mentioned, have you ever used any prescription or over the counter drugs, or natural remedies: what kind, for what conditions, when used?

drug/remedy/tx	condition (#)	approximate dates of use
________________	____________	______________________
________________	____________	______________________
________________	____________	______________________
________________	____________	______________________
________________	____________	______________________

47. During the past 12 months, have you been at bed rest due to any disease or injury for at least one week?
1 no
2 yes, _________weeks, explain:________________

LIFESTYLE ISSUES

Smoking History

94. Do you smoke or have you at some time smoked regularly (daily or almost daily)?
   1 no (go to 104)
   2 yes
   3 yes, but < 100 cigarettes (go to 104)

95. If yes, do you still smoke regularly?
   1 no 2 yes

96. How old were you when you began smoking regularly?
   _________ - years

97. If you no longer smoke regularly, how old were you when you stopped?
   _________ - years

98. How many cigarettes did you smoke on average per day before you stopped?
   1 less than 5 cigarettes
   2 5-9 cigarettes
   3 10-14 cigarettes
   4 15-19 cigarettes
   5 20-24 cigarettes
   6 25-39 cigarettes
   7 over 40 cigarettes

99. If you still smoke regularly, how many cigarettes do you smoke daily on average?
   1 less than 5 cigarettes
   2 5-9 cigarettes
   3 10-14 cigarettes
   4 15-19 cigarettes
   5 20-24 cigarettes
   6 25-39 cigarettes
   7 over 40 cigarettes

100. What is the trade name of the cigarettes you smoke (smoked) _________________.

101. How many cigars and cigarillos do you smoke per week?
   1 none
   2 less than 3 per day
   3 3-9 per day

4 10-19 per day
5 over 20 per day

102. How many packs of pipe tobacco do you smoke per week?

1 none
2 less than a half pack
3 half to a pack and a half
4 2-3 packs
5 more than 4 packs

Alcohol Consumption

104. How much of the following alcoholic beverages do you drink on average?

Beer

1 never
2 less than a bottle a week
3 1-4 bottles a week
4 5-12 bottles a week
5 13-24 bottles a week
6 25-47 bottles a week
7 more than 48 bottles a week

Wine or other mild alcoholic beverages

1 never
2 less than a glass a week
3 a glass to 4 glasses a week
4 1-2 bottles a week
5 3-4 bottles a week
6 5-9 bottles a week
7 more than 10 bottles a week

Hard liquor

1 never
2 less than a half bottle per month
3 a half bottle to a bottle and a half per month
4 2-3 bottles a month
5 4-9 bottles a month
6 10-19 bottles a month
7 more than 20 bottles month

105. How often do you use alcohol? Which of the following alternatives best describes your use of beer, wine, and hard liquor? (circle answer below for each)

	Never	On less than two days a month	On 3-8 days a month	On 9-16 days a month	Over 16 days a month
Beer	1	2	3	4	5
Wine	1	2	3	4	5
Liquor	1	2	3	4	5

---

107. On average, how many cups of coffee do you drink each day? _____ cups/day

108. How many years have you been drinking coffee regularly? __________ years

109. On average, how many cups tea do you drink each day? ___________ cups/day

110. How many years have you been drinking tea regularly?___________years

Questions concerning use of milk, sour milk and cheese

111. How much do you use per day of milk, sour milk, whole milk or cheese all together?
(1 glass=2 dl) ______________glasses

112. If you use milk products in liquid form, what type of products do you use?
1 Low fat products (ie skim milk)
2 Middle (ie 2% milk)
3 High (ie whole milk)

113. How much cheese do you eat per day? (Edam, etc.)
(1 slice= 15g) _____________slices

114. How much soft cheese do you eat per day? (cottage cheese, home cheese, etc)?
(1 spoon= 20g) ________________spoons (spoon = 20g)

115. Has the situation been the same all your life?
1 Earlier I used more
2 Situation is the same
3 Earlier I used less

116. possible explanation
(when,why):____

____

Exercise History
(ball games, track and field, endurance, power sports, etc.)

117. Before 12 years of age, were there any conditions that caused you to be significantly less or more physically active than other young children?

1 no
2 yes

118. If yes, were you more or less active?
Explain:(more = 1, less = 2, why)________________________________

____

____

119.1
Age span (yrs) from age______to age_______
Exercise (classification): ____
Competition level sport, best results (classification):_________________________
Was your participation: 1 year-round 2 seasonal, _____ mos
Frequency (x/week) ______________________
Duration (min./x) ______________________
Intensity (1=light, 2=medium 3=heavy)__________________________________
Related injuries 1 no 2 yes
If yes, explain: ____

119.2 Repeat…..

120. Who currently engages in more physical exercise/sport, you or your twin?

1 you
2 your twin
3 unsure

Explain: ____

____

121. Over your lifetime, who has engaged in more exercise/sport, you or your twin?

1 you
2 your twin

3 unsure
Explain: ____

____

122. During the past 3 days, have you done some extremely physically hard work or training?
1 no
2 yes
If yes, what? ____

____

OTHER LEISURE TIME PHYSICAL ACTIVITY HISTORY
(other ADL, ie. building your home, hiking, hunting, forest work)

124.1
Age span (yrs) from age______to age_______
Activity (category)____
Was your participation: 1 year-round 2 seasonal, _____m[illegible]
Frequency (x/wk) _____________
Duration (min/x) _____________
Intensity _____________(1=light, 2=medium, 3=heavy)
Related injuries?
1 no 2 yes
If yes, what?____

____

____

124.2 Repeat

125. Who currently engages in more non-exercise/sport leisure activities, you or your twin?
1 you
2 your twin
3 unsure
Explain:

____

126. Over your lifetime, who has engaged in more non-exercise/sport leisure activities, you or your twin?
1 you
2 your twin
3 unsure

Explain:

____

WORK HISTORY

131. Did you do physically hard work when you were under 20?
1 no 2 yes

132. If yes, what ____
____

133. What kind of work did you do during your schooling?
1 not heavy with regard to back
2 possibly heavy
3 clearly heavy,
Explain ____________________________________
____

134.1 **Job Title** ____________________________

Job description ____________________________________

Job Category (1-5) ____________________________

Age span (yrs) from age________ to age________
1 year-round

2 seasonal, _____ mos.

Sitting (not including in car) hrs/day __________

Lifting :
(most common weight x frequency) _______kg x ________/hr/day/wk/mo
(heaviest weight, at least 1 x/ mo) _______kg x ________/hr/day/wk/mo

Standing/walking ______hrs/day
Total time in sitting, driving, bend/twist, + standing/walking = _______hrs/day
(Estimate total. Does this seem reasonable?)

What positions/activities make-up the remainder of your work day?____________________________________

Work hours per week:(___hrs x___days) = _______hrs/wk

Commute time:________(minutes to and from work)
Mode of transportation: 1 walk 2 bicycle 3 motor vehicle 4 other
Type of vehicle(model yr.): ____________________________________

Injuries?
1 no 2 yes
If yes, type? when?____
____

Sudden back pain/injuries?
1 no 2 yes
If yes,explain (type,when)____
____

134.2 Repeat...

Comparison of work-related factors between twins
(These work-related questions are related to your present or most recent fob.)

135. In your current or most recent job, who spends more time sitting?

1 you
2 your twin
3 unsure

136. Has this been the case throughout most of your working lives?
1 no
2 yes

137. If no, who has spent more time sitting at work overall?
1 you
2 your twin
3 unsure

141. In your current or most recent job, who does more lifting?
1 you
2 your twin
3 unsure

142. Has this been the case throughout most of your working lives?
1 no
2 yes

143. If no, who has done more lifting at work overall?
1 you
2 your twin
3 unsure

147. Overall, who has had the most physically demanding work over the years, you or your twin?

1 you
2 your twin
3 unsure
Explain:

____
____

148. What are the biggest differences between you and your twin, in terms of your life styles or life circumstances?

____
____
____

Other
comments:____
____

Misc. notes to interviewers (including phone number for contact if needed)

134.1 Work type classification

1. Mainly sedentary

2. Light, mainly walking and standing (lifting <10 kg)

3. Varying including some lifting (lifting <35 kg)

4. Varying including some lifting (lifting >35 kg)

5. Heavy physical work

CODING FOR SPECIFIC QUESTIONNAIRE ITEMS:

119.1 Type of Exercise

1. Ball games
    1.1 Soccer
    1.2 Volleyball
    1.3 Ice hockey/ Sahly
    1.4 Base ball
    1.5 Basket ball
    1.6 Tennis
    1.7 Squash

    1.9 Others____________________

2. Endurance events
    2.1 Running/jogging
    2.2 Cross country skiing
    2.3 Cycling
    2.4 Orienteering
    2.5 Walking
    2.9 Others__________________

3. Gymnastics

4. Swimming

5. Down hill skiing
6. Power sport
    6.1 Body building
    6.2 Weight lifting
    6.9 Others_____________________

11. Bowling

12. Golf

13. Aerobics

14. Home gymnastics

19. Others____

119.1 (cont.) Competitive events

1. Ball games
    1.1 Soccer
    1.2 Volley ball
    1.3 Ice hockey
    1.4 Base ball
    1.5 Basket ball
    1.6 Tennis
    1.7 Squash
    1.9 Others
2. Endurance sports
    2.1 Running 1500 m and more
    2.2 Cross country skiing
    2.3 Cycling
    2.4 Orienteering

    - 2.9 Others________________
3. Gymnastics
4. Swimming
5. Down hill skiing
6. Power sports
    - 6.1 Body building
    - 6.2 Weight lifting
    - 6.9 Others____________________
7. Combative sports
    - 7.1 Boxing
    - 7.2 Wrestling
    - 7.9 Others_____________________
8. Speed sports
    - 8.1 100-1000 m
    - 8.2 Hurdles
    - 8.9 Others_________________
9. Field sports (throwing events)

    ____

10. Jumping events

    ____

11. Bowling
12. Golf

19. Others

____

124.1 Free time activities

1. Working at summer cottage/gardening/working on the house

2. Hiking/picking berries

3. Hunting

4. Fishing

5. Building own house

6. Forest work

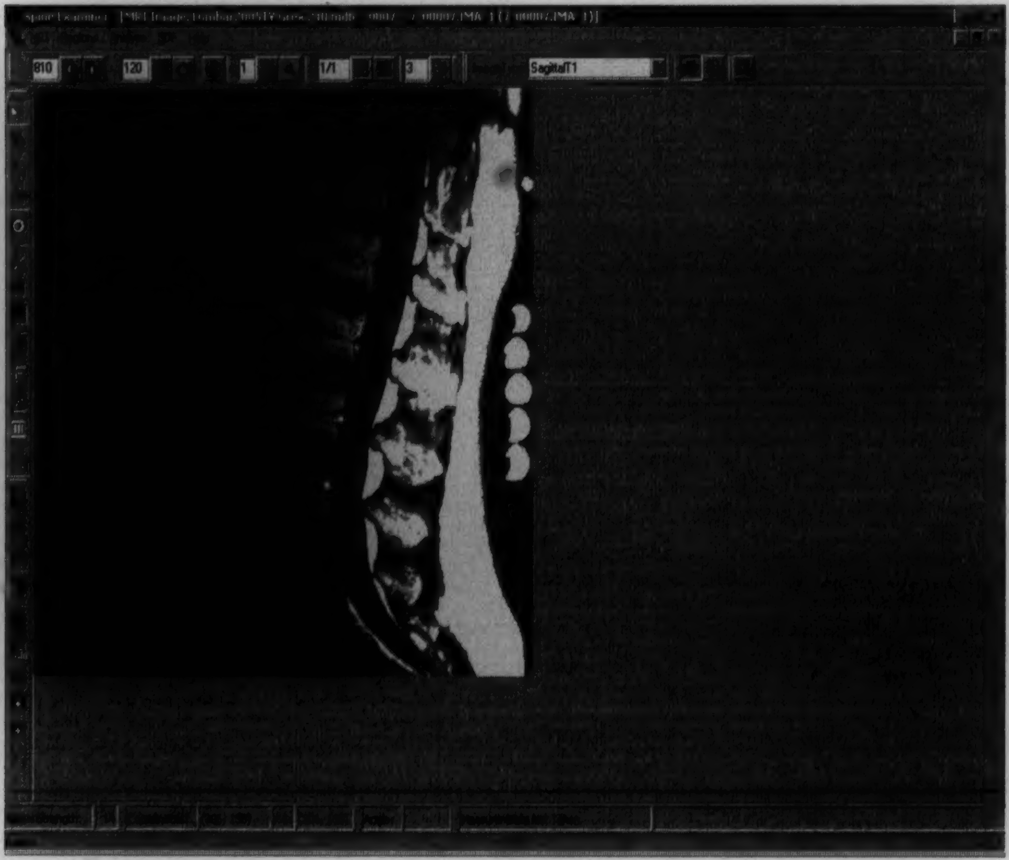
7. Dancing

19. Others

APPENDIX B: MANUAL SEGMENTATION PROCEDURE

Outlining regions for measures at the L1-L5 vertebrae

The contour of the lumbar discs and vertebrae has been manually segmented in order to derive measures of the vertebral body. The sagittal T1 image was first segmented, as it displays the best overall contrast between the structures of interest (the contrast between the bone or fat and the other tissues is excellent and therefore the fat and bone are best seen on T1 sequence). The image was zoomed by a factor of 5. The brightness was adjusted to a value of 120, while the contrast was adjusted as necessary.



Segmentation of the anterior vertebral wall and disc:

The manual segmentation began first by segmenting the anterior wall of the vertebrae and disc from the anterior longitudinal ligament by a vertical line. The first point was placed as high on the image as possible at the border between the bone and the anterior longitudinal ligament. The line was continued by adding points to follow the contour of the vertebrae and discs. The last point on the vertebral contour was placed at the lower limit of the anterior wall. A point was placed at the level where the endplate would cross the disc contour. This was repeated for each disc and vertebra until reaching the upper limit of S1. If an osteophyte formed near the corners of the vertebra, its area was included within the vertebral contour.

Segmentation of the posterior vertebral wall and disc:

The posterior vertebral wall was manually segmented from the posterior longitudinal ligament or sub-membranous space by creating a vertical line starting at the top of the T1 image and continuing at the disc level. The posterior longitudinal ligament was segmented from the soft tissue or CSF fluid located at the back of the spine.

Segmentation of upper and vertebral endplate/disc interfaces

The upper and lower endplates of each vertebra were segmented by creating horizontal lines along the endplates. The last horizontal segmentation line was placed by following the lower L5 endplate. Each line began outside of the vertebra so that the horizontal line crossed the vertical line segmenting the anterior vertebral wall and disc only once.

Adjustment of segmentation using T2 -and T1-weighted images

The regions of interest, corresponding to the areas enclosed within the vertical and horizontal segmentation lines, were copied from the T1 image on the corresponding T2-weighted image to adjust the CSF. On the T2-weighted image, the contrast between the CSF and the other tissues was excellent and the posterior vertical segmentation line was adjusted taking advantage of the improved constrast on this sequence.

The regions of interest were then copied onto the corresponding T1-weighted images to finish adjusting the segmented outline. The bone, the osteophytes and endplate sclerosis were adjusted on this sequence taking advantage of the improved contrast using T1.

APPENDIX C: INTRA AND INTER- RATER RELIABILITY FOR MRI MEASURES

INTRA-RATER RELIABILITY COEFFICIENTS

Correlations of repeat measurements of vertebral body (CJH) area (AS), signal intensity (AI) or adjacent signal intensity of CSF (452) for each level (obtained by the same rater, myself: O = Oana, S = Smaranda).

L1 LEVEL

Correlations

		OL1AI452	OL1AICJH	OL1VAR	OL1ASCJH	SL1AI452	SL1AICJH	SL1AIVAR	SL1ASCJH
OL1AI452	Pearson Correlation	1	.829**	-.467*	.331	.929**	.820**	-.477*	.289
	Sig. (2-tailed)	.	.000	.012	.085	.000	.000	.010	.136
	N	28	28	28	28	28	28	28	28
OL1AICJH	Pearson Correlation	.829**	1	-.514**	.196	.942**	.999**	-.577**	.084
	Sig. (2-tailed)	.000	.	.005	.319	.000	.000	.001	.671
	N	28	28	28	28	28	28	28	28
OL1VAR	Pearson Correlation	-.467*	-.514**	1	-.030	-.515**	-.500**	.927**	-.126
	Sig. (2-tailed)	.012	.005	.	.880	.005	.007	.000	.522
	N	28	28	28	28	28	28	28	28
OL1ASCJH	Pearson Correlation	.331	.196	-.030	1	.213	.187	.099	.941**
	Sig. (2-tailed)	.085	.319	.880	.	.275	.340	.616	.000
	N	28	28	28	28	28	28	28	28
SL1AI452	Pearson Correlation	.929**	.942**	-.515**	.213	1	.942**	-.540**	.170
	Sig. (2-tailed)	.000	.000	.005	.275	.	.000	.002	.377
	N	28	28	28	28	29	29	29	29
SL1AICJH	Pearson Correlation	.820**	.999**	-.500**	.187	.942**	1	-.579**	.106
	Sig. (2-tailed)	.000	.000	.007	.340	.000	.	.001	.583
	N	28	28	28	28	29	29	29	29
SL1AIVAR	Pearson Correlation	-.477*	-.577**	.927**	.099	-.540**	-.579**	1	.048
	Sig. (2-tailed)	.010	.001	.000	.616	.002	.001	.	.803
	N	28	28	28	28	29	29	29	29
SL1ASCJH	Pearson Correlation	.289	.084	-.126	.941**	.170	.106	.048	1
	Sig. (2-tailed)	.136	.671	.522	.000	.377	.583	.803	.
	N	28	28	28	28	29	29	29	29

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

L2 LEVEL

Correlations

		OL2AI452	OL2AICJH	OL2VAR	OL2ASCJH	SL2AI452	SL2AICJH	SL2AIVAR	SL2ASCJH
OL2AI452	Pearson Correlation	1	.847**	-.455*	.145	.944**	.851**	-.431*	.176
	Sig. (2-tailed)	.	.000	.013	.453	.000	.000	.019	.361
	N	29	29	29	29	29	29	29	29
OL2AICJH	Pearson Correlation	.847**	1	-.512**	.056	.933**	.999**	-.510**	.186
	Sig. (2-tailed)	.000	.	.005	.773	.000	.000	.005	.334
	N	29	29	29	29	29	29	29	29
OL2VAR	Pearson Correlation	-.455*	-.512**	1	.061	-.455*	-.498**	.909**	-.140
	Sig. (2-tailed)	.013	.005	.	.753	.013	.006	.000	.469
	N	29	29	29	29	29	29	29	29
OL2ASCJH	Pearson Correlation	.145	.056	.061	1	.052	.065	-.076	.880**
	Sig. (2-tailed)	.453	.773	.753	.	.790	.737	.695	.000
	N	29	29	29	29	29	29	29	29
SL2AI452	Pearson Correlation	.944**	.933**	-.455*	.052	1	.933**	-.430*	.154
	Sig. (2-tailed)	.000	.000	.013	.790	.	.000	.020	.424
	N	29	29	29	29	29	29	29	29
SL2AICJH	Pearson Correlation	.851**	.999**	-.498**	.065	.933**	1	-.514**	.178
	Sig. (2-tailed)	.000	.000	.006	.737	.000	.	.004	.356
	N	29	29	29	29	29	29	29	29
SL2AIVAR	Pearson Correlation	-.431*	-.510**	.909**	-.076	-.430*	-.514**	1	-.157
	Sig. (2-tailed)	.019	.005	.000	.695	.020	.004	.	.416
	N	29	29	29	29	29	29	29	29
SL2ASCJH	Pearson Correlation	.176	.186	-.140	.880**	.154	.178	-.157	1
	Sig. (2-tailed)	.361	.334	.469	.000	.424	.356	.416	.
	N	29	29	29	29	29	29	29	29

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

L3 LEVEL

Correlations

		OL3AI452	OL3AICJH	OL3VAR	OL3ASCJH	SL3AI452	SL3AICJH	SL3AIVAR	SL3ASCJH
OL3AI452	Pearson Correlation	1	.828**	-.202	.122	.958**	.831**	-.221	.050
	Sig. (2-tailed)	.	.000	.294	.528	.000	.000	.249	.798
	N	29	29	29	29	29	29	29	29
OL3AICJH	Pearson Correlation	.828**	1	-.379*	.060	.849**	.998**	-.378*	.122
	Sig. (2-tailed)	.000	.	.043	.756	.000	.000	.043	.529
	N	29	29	29	29	29	29	29	29
OL3VAR	Pearson Correlation	-.202	-.379*	1	.177	-.334	-.378*	.983**	.096
	Sig. (2-tailed)	.294	.043	.	.360	.077	.043	.000	.621
	N	29	29	29	29	29	29	29	29
OL3ASCJH	Pearson Correlation	.122	.060	.177	1	.067	.068	.163	.854**
	Sig. (2-tailed)	.528	.756	.360	.	.729	.727	.397	.000
	N	29	29	29	29	29	29	29	29
SL3AI452	Pearson Correlation	.958**	.849**	-.334	.067	1	.850**	-.338	.008
	Sig. (2-tailed)	.000	.000	.077	.729	.	.000	.073	.966
	N	29	29	29	29	29	29	29	29
SL3AICJH	Pearson Correlation	.831**	.998**	-.378*	.068	.850**	1	-.385*	.113
	Sig. (2-tailed)	.000	.000	.043	.727	.000	.	.039	.559
	N	29	29	29	29	29	29	29	29
SL3AIVAR	Pearson Correlation	-.221	-.378*	.983**	.163	-.338	-.385*	1	.141
	Sig. (2-tailed)	.249	.043	.000	.397	.073	.039	.	.467
	N	29	29	29	29	29	29	29	29
SL3ASCJH	Pearson Correlation	.050	.122	.096	.854**	.008	.113	.141	1
	Sig. (2-tailed)	.798	.529	.621	.000	.966	.559	.467	.
	N	29	29	29	29	29	29	29	29

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

L4 LEVEL

Correlations

		OL4AI452	OL4AICJH	OL4VAR	OL4ASCJH	SL4AI452	SL4AICJH	SL4AIVAR	SL4ASCJH
OL4AI452	Pearson Correlation	1	.884**	-.318	.118	.817**	.882**	-.316	.133
	Sig. (2-tailed)	.	.000	.092	.543	.000	.000	.095	.493
	N	29	29	29	29	29	29	29	29
OL4AICJH	Pearson Correlation	.884**	1	-.497**	.082	.546**	.998**	-.493**	.138
	Sig. (2-tailed)	.000	.	.006	.674	.002	.000	.007	.475
	N	29	29	29	29	29	29	29	29
OL4VAR	Pearson Correlation	-.318	-.497**	1	-.020	.078	-.480**	.992**	-.051
	Sig. (2-tailed)	.092	.006	.	.918	.689	.008	.000	.792
	N	29	29	29	29	29	29	29	29
OL4ASCJH	Pearson Correlation	.118	.082	-.020	1	-.065	.077	-.012	.923**
	Sig. (2-tailed)	.543	.674	.918	.	.737	.691	.949	.000
	N	29	29	29	29	29	29	29	29
SL4AI452	Pearson Correlation	.817**	.546**	.078	-.065	1	.559**	.059	-.092
	Sig. (2-tailed)	.000	.002	.689	.737	.	.002	.759	.635
	N	29	29	29	29	29	29	29	29
SL4AICJH	Pearson Correlation	.882**	.998**	-.480**	.077	.559**	1	-.480**	.135
	Sig. (2-tailed)	.000	.000	.008	.691	.002	.	.008	.486
	N	29	29	29	29	29	29	29	29
SL4AIVAR	Pearson Correlation	-.316	-.493**	.992**	-.012	.059	-.480**	1	-.027
	Sig. (2-tailed)	.095	.007	.000	.949	.759	.008	.	.891
	N	29	29	29	29	29	29	29	29
SL4ASCJH	Pearson Correlation	.133	.138	-.051	.923**	-.092	.135	-.027	1
	Sig. (2-tailed)	.493	.475	.792	.000	.635	.486	.891	.
	N	29	29	29	29	29	29	29	29

**. Correlation is significant at the 0.01 level (2-tailed).

L5 LEVEL

Correlations

		OL5AI452	OL5AICJH	OL5VAR	OL5ASCJH	SL5AI452	SL5AICJH	SL5AIVAR	SL5ASCJH
OL5AI452	Pearson Correlation	1	.860**	-.411*	-.001	.882**	.861**	-.413*	-.012
	Sig. (2-tailed)	.	.000	.027	.996	.000	.000	.026	.951
	N	29	29	29	29	29	29	29	29
OL5AICJH	Pearson Correlation	.860**	1	-.363	.153	.687**	.998**	-.365	.110
	Sig. (2-tailed)	.000	.	.053	.428	.000	.000	.051	.570
	N	29	29	29	29	29	29	29	29
OL5VAR	Pearson Correlation	-.411*	-.363	1	.257	-.249	-.366	.964**	.197
	Sig. (2-tailed)	.027	.053	.	.179	.193	.051	.000	.305
	N	29	29	29	29	29	29	29	29
OL5ASCJH	Pearson Correlation	-.001	.153	.257	1	-.136	.155	.272	.930**
	Sig. (2-tailed)	.996	.428	.179	.	.483	.423	.153	.000
	N	29	29	29	29	29	29	29	29
SL5AI452	Pearson Correlation	.882**	.687**	-.249	-.136	1	.697**	-.260	-.145
	Sig. (2-tailed)	.000	.000	.193	.483	.	.000	.174	.454
	N	29	29	29	29	29	29	29	29
SL5AICJH	Pearson Correlation	.861**	.998**	-.366	.155	.697**	1	-.372*	.109
	Sig. (2-tailed)	.000	.000	.051	.423	.000	.	.047	.575
	N	29	29	29	29	29	29	29	29
SL5AIVAR	Pearson Correlation	-.413*	-.365	.964**	.272	-.260	-.372*	1	.283
	Sig. (2-tailed)	.026	.051	.000	.153	.174	.047	.	.137
	N	29	29	29	29	29	29	29	29
SL5ASCJH	Pearson Correlation	-.012	.110	.197	.930**	-.145	.109	.283	1
	Sig. (2-tailed)	.951	.570	.305	.000	.454	.575	.137	.
	N	29	29	29	29	29	29	29	29

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

INTER-RATER RELIABILITY COEFFICIENTS:

Correlations between area (AS) or signal intensity (AI) measurements for vertebral body (CJH) or CSF (452) for each level, by rater (T= rater 1, supervisor and S= rater 2, myself)

L1 LEVEL

Correlations

		TL1AI452	TL1AICJH	TL1AIVAR	TL1ASCJH	SL1AI452	SL1AICJH	SL1AIVAR	SL1ASVAR
TL1AI452	Pearson Correlation	1	.923**	-.424*	.245	.993**	.923**	-.475**	.182
	Sig. (2-tailed)	.	.000	.019	.192	.000	.000	.008	.335
	N	30	30	30	30	30	30	30	30
TL1AICJH	Pearson Correlation	.923**	1	-.526**	.216	.939**	1.000**	-.565**	.126
	Sig. (2-tailed)	.000	.	.003	.252	.000	.000	.001	.506
	N	30	30	30	30	30	30	30	30
TL1AIVAR	Pearson Correlation	-.424*	-.526**	1	-.058	-.433*	-.526**	.963**	-.066
	Sig. (2-tailed)	.019	.003	.	.760	.017	.003	.000	.729
	N	30	30	30	30	30	30	30	30
TL1ASCJH	Pearson Correlation	.245	.216	-.058	1	.245	.220	-.060	.932**
	Sig. (2-tailed)	.192	.252	.760	.	.193	.243	.753	.000
	N	30	30	30	30	30	30	30	30
SL1AI452	Pearson Correlation	.993**	.939**	-.433*	.245	1	.938**	-.479**	.180
	Sig. (2-tailed)	.000	.000	.017	.193	.	.000	.007	.340
	N	30	30	30	30	30	30	30	30
SL1AICJH	Pearson Correlation	.923**	1.000**	-.526**	.220	.938**	1	-.569**	.126
	Sig. (2-tailed)	.000	.000	.003	.243	.000	.	.001	.507
	N	30	30	30	30	30	30	30	30
SL1AIVAR	Pearson Correlation	-.475**	-.565**	.963**	-.060	-.479**	-.569**	1	-.033
	Sig. (2-tailed)	.008	.001	.000	.753	.007	.001	.	.864
	N	30	30	30	30	30	30	30	30
SL1ASVAR	Pearson Correlation	.182	.126	-.066	.932**	.180	.126	-.033	1
	Sig. (2-tailed)	.335	.506	.729	.000	.340	.507	.864	.
	N	30	30	30	30	30	30	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

L2 LEVEL

Correlations

		TL2AI452	TL2AICJH	TL2AIVAR	TL2ASCJH	SL2AI452	SL2AICJH	SL2AIVAR	SL2ASCJH
TL2AI452	Pearson Correlation	1	.917**	-.516**	.084	.986**	.913**	-.476**	.080
	Sig. (2-tailed)	.	.000	.004	.658	.000	.000	.008	.674
	N	30	30	30	30	30	30	30	30
TL2AICJH	Pearson Correlation	.917**	1	-.572**	.168	.929**	.999**	-.564**	.121
	Sig. (2-tailed)	.000	.	.001	.376	.000	.000	.001	.526
	N	30	30	30	30	30	30	30	30
TL2AIVAR	Pearson Correlation	-.516**	-.572**	1	-.109	-.476**	-.567**	.971**	-.081
	Sig. (2-tailed)	.004	.001	.	.567	.008	.001	.000	.669
	N	30	30	30	30	30	30	30	30
TL2ASCJH	Pearson Correlation	.084	.168	-.109	1	.132	.165	-.131	.943**
	Sig. (2-tailed)	.658	.376	.567	.	.488	.383	.492	.000
	N	30	30	30	30	30	30	30	30
SL2AI452	Pearson Correlation	.986**	.929**	-.476**	.132	1	.927**	-.449*	.119
	Sig. (2-tailed)	.000	.000	.008	.488	.	.000	.013	.530
	N	30	30	30	30	30	30	30	30
SL2AICJH	Pearson Correlation	.913**	.999**	-.567**	.165	.927**	1	-.565**	.110
	Sig. (2-tailed)	.000	.000	.001	.383	.000	.	.001	.562
	N	30	30	30	30	30	30	30	30
SL2AIVAR	Pearson Correlation	-.476**	-.564**	.971**	-.131	-.449*	-.565**	1	-.055
	Sig. (2-tailed)	.008	.001	.000	.492	.013	.001	.	.773
	N	30	30	30	30	30	30	30	30
SL2ASCJH	Pearson Correlation	.080	.121	-.081	.943**	.119	.110	-.055	1
	Sig. (2-tailed)	.674	.526	.669	.000	.530	.562	.773	.
	N	30	30	30	30	30	30	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

L3 LEVEL

Correlations

		TL3AI452	TL3AICJH	TL3AIVAR	TL3ASCJH	SL3AI452	SL3AICJH	SL3AIVAR	SL3ASCJH
TL3AI452	Pearson Correlation	1	.767**	-.308	.099	.924**	.767**	-.299	.062
	Sig. (2-tailed)	.	.000	.097	.601	.000	.000	.108	.745
	N	30	30	30	30	30	30	30	30
TL3AICJH	Pearson Correlation	.767**	1	-.419*	.119	.824**	.998**	-.419*	.017
	Sig. (2-tailed)	.000	.	.021	.531	.000	.000	.021	.930
	N	30	30	30	30	30	30	30	30
TL3AIVAR	Pearson Correlation	-.308	-.419*	1	.152	-.358	-.408*	.984**	.145
	Sig. (2-tailed)	.097	.021	.	.422	.052	.025	.000	.446
	N	30	30	30	30	30	30	30	30
TL3ASCJH	Pearson Correlation	.099	.119	.152	1	.004	.130	.168	.952**
	Sig. (2-tailed)	.601	.531	.422	.	.982	.494	.374	.000
	N	30	30	30	30	30	30	30	30
SL3AI452	Pearson Correlation	.924**	.824**	-.358	.004	1	.824**	-.342	-.006
	Sig. (2-tailed)	.000	.000	.052	.982	.	.000	.064	.974
	N	30	30	30	30	30	30	30	30
SL3AICJH	Pearson Correlation	.767**	.998**	-.408*	.130	.824**	1	-.412*	.026
	Sig. (2-tailed)	.000	.000	.025	.494	.000	.	.024	.891
	N	30	30	30	30	30	30	30	30
SL3AIVAR	Pearson Correlation	-.299	-.419*	.984**	.168	-.342	-.412*	1	.176
	Sig. (2-tailed)	.108	.021	.000	.374	.064	.024	.	.353
	N	30	30	30	30	30	30	30	30
SL3ASCJH	Pearson Correlation	.062	.017	.145	.952**	-.006	.026	.176	1
	Sig. (2-tailed)	.745	.930	.446	.000	.974	.891	.353	.
	N	30	30	30	30	30	30	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

L4 LEVEL

Correlations

		TL4AI452	TL4AICJH	TL4AIVAR	TL4ASCJH	SL4AI452	SL4AICJH	SL4AIVAR	SL4ASCJH
TL4AI452	Pearson Correlation	1	.601**	.020	.138	.874**	.611**	.002	.030
	Sig. (2-tailed)	.	.000	.916	.468	.000	.000	.993	.874
	N	30	30	30	30	30	30	30	30
TL4AICJH	Pearson Correlation	.601**	1	-.451*	.172	.536**	.999**	-.465**	.086
	Sig. (2-tailed)	.000	.	.012	.363	.002	.000	.010	.650
	N	30	30	30	30	30	30	30	30
TL4AIVAR	Pearson Correlation	.020	-.451*	1	.100	.071	-.430*	.992**	-.040
	Sig. (2-tailed)	.916	.012	.	.600	.709	.018	.000	.832
	N	30	30	30	30	30	30	30	30
TL4ASCJH	Pearson Correlation	.138	.172	.100	1	.018	.199	.081	.904**
	Sig. (2-tailed)	.468	.363	.600	.	.924	.291	.671	.000
	N	30	30	30	30	30	30	30	30
SL4AI452	Pearson Correlation	.874**	.536**	.071	.018	1	.544**	.061	-.094
	Sig. (2-tailed)	.000	.002	.709	.924	.	.002	.750	.623
	N	30	30	30	30	30	30	30	30
SL4AICJH	Pearson Correlation	.611**	.999**	-.430*	.199	.544**	1	-.446*	.107
	Sig. (2-tailed)	.000	.000	.018	.291	.002	.	.013	.575
	N	30	30	30	30	30	30	30	30
SL4AIVAR	Pearson Correlation	.002	-.465**	.992**	.081	.061	-.446*	1	-.031
	Sig. (2-tailed)	.993	.010	.000	.671	.750	.013	.	.871
	N	30	30	30	30	30	30	30	30
SL4ASCJH	Pearson Correlation	.030	.086	-.040	.904**	-.094	.107	-.031	1
	Sig. (2-tailed)	.874	.650	.832	.000	.623	.575	.871	.
	N	30	30	30	30	30	30	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

L5 LEVEL

Correlations

		TL5AI452	TL5AICJH	TL5AIVAR	TL5ASCJH	SL5AI452	SL5AICJH	SL5AIVAR	SL5ASCJH
TL5AI452	Pearson Correlation	1	.771**	-.296	-.095	.882**	.769**	-.322	-.182
	Sig. (2-tailed)	.	.000	.112	.618	.000	.000	.083	.334
	N	30	30	30	30	30	30	30	30
TL5AICJH	Pearson Correlation	.771**	1	-.373*	.194	.697**	.999**	-.374*	.082
	Sig. (2-tailed)	.000	.	.043	.304	.000	.000	.042	.666
	N	30	30	30	30	30	30	30	30
TL5AIVAR	Pearson Correlation	-.296	-.373*	1	.359	-.234	-.365*	.963**	.313
	Sig. (2-tailed)	.112	.043	.	.052	.214	.048	.000	.092
	N	30	30	30	30	30	30	30	30
TL5ASCJH	Pearson Correlation	-.095	.194	.359	1	-.074	.210	.293	.909**
	Sig. (2-tailed)	.618	.304	.052	.	.699	.264	.116	.000
	N	30	30	30	30	30	30	30	30
SL5AI452	Pearson Correlation	.882**	.697**	-.234	-.074	1	.692**	-.262	-.147
	Sig. (2-tailed)	.000	.000	.214	.699	.	.000	.163	.437
	N	30	30	30	30	30	30	30	30
SL5AICJH	Pearson Correlation	.769**	.999**	-.365*	.210	.692**	1	-.369*	.095
	Sig. (2-tailed)	.000	.000	.048	.264	.000	.	.045	.617
	N	30	30	30	30	30	30	30	30
SL5AIVAR	Pearson Correlation	-.322	-.374*	.963**	.293	-.262	-.369*	1	.284
	Sig. (2-tailed)	.083	.042	.000	.116	.163	.045	.	.128
	N	30	30	30	30	30	30	30	30
SL5ASCJH	Pearson Correlation	-.182	.082	.313	.909**	-.147	.095	.284	1
	Sig. (2-tailed)	.334	.666	.092	.000	.437	.617	.128	.
	N	30	30	30	30	30	30	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX D: ACRONYMS USED IN THE TEXT

National Institutes of Health (NIH)
World Health Organization (WHO)
dual-energy x-ray absorptiometry (DXA)
bone mineral density (BMD)
body mass index (BMI)
Quantitative computerized tomography (QCT)
magnetic resonance imaging (MRI)
quantitative ultrasound (QUS)
cerebrospinal fluid (CSF)

Monozygotic (MZ) twins
dizygotic (DZ) twins
additive effects of genes taken singly and added over multiple loci (A),
dominance effects of genes interacting within loci (D),
common environment shared by family members (C)
unique environment not shared by family members (E)
Akaike's information criterion (AIC)
Additive genetic correlation (r_a)
Unique environmental correlation (r_e)
Proportion of total genetic variation in marker variable explained by common genetic variation with the other phenotype in model (Pa)
Proportion of total unshared environmental variation in marker variable explained by common unshared environmental variation with the other phenotype in model (Pe)
heritability estimates (H^2)
least significant change (LSC)

estrogen receptor alfa/beta (ERα/ERβ)
androgen receptor (AR)
vitamin D receptor gene (VDR)

collagen type I alfa 1/2 gene (COL1A1/ COL1A2)
insulin growth factor I gene (IGF-I)
interleukin 6(IL6)
transforming growth factor β1(TGFβ1)
apoprotein E (APOE)

osteocalcin (OC)
amino- terminal propeptide of type I collagen (PINP)
carboxy- terminal propeptide of type I collagen (PICP)
amino-terminal type I collagen peptide (NTx)
carboxy-terminal type I collagen telopeptide (CTx)
carboxy-terminal telopeptide of type I collagen (ICTP)
hydroxyproline (Hyp)
total alkaline phosphatase (ALP)
bone specific alkaline phosphatase (BALP)
bone specific alkaline phosphatase, BAP
bone specific alkaline phosphatase (BSAP)
pyridinoline (Pyr/ Pyd)
deoxypyridinoline (dPyr/ Dpd)
tartrate-resistant acid phosphatase (TRAP)
calcium (Ca)
hydroxyline glycosides (GHYL)